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OFFICE OF THE DIRECTOR

OFFICE OF THE DIRECTOR
Summary Statement

The twelve years the Institute has been in existence have brought enormous changes in the field of environmental health; as important, they have brought major changes in the public's perceptions and expectations of the field.

In response to public expectations, the first seven years of this decade saw a great outpouring of legislation aimed at strengthening ongoing efforts and protecting the public from the adverse by-products of technological development. Historically most environmental health legislation was directed at control of toxins after their introduction into the environment. However, with passage of the Toxic Substances Control Act in the Fall of 1976, Congress established as public policy the concept that we should take action to prevent possibly hazardous compounds from being introduced into commerce. Thus, a new era in environmental health began. While many regarded passage of the Toxic Substances Control Act as the completion of a long and difficult legislative struggle, we can now see that it was really just a beginning. It was a beginning, the full impact of which we will not understand fully for several years.

What is clear is that, as opposed to most legislative events, the initiative has not truly shifted to the Executive; rather Congress and its support agencies (General Accounting Office, Office of Technology Assessment and the Congressional Research Service) are taking a real and continuing interest in the performance of the Executive Branch in its implementation of this legislation.

In addition, in response to their concern regarding possible inadequate Executive Branch coordination, both Congress and the President have continued to create additional coordinating groups. The Institute is currently tracking activities of approximately fifteen such groups, including the Toxic Substances Strategy Committee and the Task Force on Environmental Cancer and Heart and Lung Disease.

It is clear from all this activity that the government has not yet developed a clear concept which can serve as the driving force for ongoing efforts and new initiatives in this field. However, the cross-cutting and pervasive nature of the problems in this area have in fact forced a significant amount of real cooperation between agencies, both national and international, and between workers in the field, regardless of where located.

It is troubling, though, that public commitment to environmental control measures so far outweighs the commitment to the development of new knowledge upon which control measures must necessarily be based. Until this imbalance is at least partially corrected we believe our control measures will be too

often based, after the fact, on exposures to human populations. In our view this is too high a cost to pay; and as we have discovered with asbestos and Kepone, it is too often payment on the installment plan, with high interest, and no final payment.

While the Institute took part in a variety of important interagency and transgovernmental efforts, many of which are discussed in the program summaries that follow, two stand out both because of their importance and because of how they came to be carried out. These are: The development of the "Report of Human Health and Environmental Effects of Increased Use of Coal Utilization" (mandated by the President in his national energy plan), and the HEW Secretary's establishment of the Asbestos Working Group, concerned with the health of asbestos workers and other individuals exposed to asbestos in other settings. Our office managed these two efforts, which had strong interagency ties and drew on the expertise of the non-governmental science community.

As a final general comment, it is important to note the growing apprehension that scientists are telling the public nothing is safe, and therefore the public can not reasonably be expected to take any action to protect itself against every possible environmental hazard. A number of respected public commentators have spoken out in this vein in recent months. Clearly development of a coherent, easily understood answer to those comments is a matter of importance for the months ahead.

It is against this backdrop of continuing legislative, executive, and public interest in environmental health that the NIEHS is carrying out its mission to provide the scientific information base, advanced scientific methodology, and trained scientific manpower to reach an understanding of the total impact of environmental factors on human health.

The year just past has seen a continuation of the evolution of the Institute's research and manpower development programs. The summaries which follow discuss in detail specific events and progress in these programs. In order to assure their continuing growth and development, as well as their responsiveness to national need, the Institute is undertaking a long-term planning effort aimed at translating into specific plans the broad needs and goals identified in the report of the Second Task Force on Research Planning in Environmental Health Science. Three sub-groups of governmental and non-governmental scientists are carrying out this effort; and they will complete their preliminary report by the end of the fiscal year. The final report will form the basis for the 1981-85 Forward Plan and other Institute planning activities in the years ahead.

EXTRAMURAL PROGRAM

OFFICE OF THE ASSOCIATE DIRECTOR FOR EXTRAMURAL PROGRAM
Summary Statement

Research Grants

During FY-1978, 436 Regular Research, Program Project, Young Investigator, and Research Career Development Award applications were reviewed, a significant increase from FY-1977. Awards were made for 133 noncompeting and 128 competing projects from the FY-1978 Continuing Resolution for a total of \$22.3 million or 60 percent of those reviewed.

Manpower Development Resources

Fiscal Year-1978 showed a continued increase in the NIEHS commitment to training. The budget for Manpower Development Resources grew from \$4.7 million in FY-1977 to \$5.3 million in FY-1978. This allowed our program to support approximately 52 institutional awards in environmental health sciences, a growth of 26 over the past year. A concomitant expansion of the individual postdoctoral fellowship program has also taken place.

A major portion (65-70 percent) of these training programs are in the critical area of environmental toxicology and represent the only federal effort of any consequence to train people in this field. At the FY-1978 level, the program supports on the order of 230 pre- and 230 postdoctoral trainees. The annual output rate is, therefore, 192 environmental health scientists, 125 of whom are toxicologists. The rest are trained in environmental pathology, environmental epidemiology, and environmental mutagenesis. These scientists represent a core of individuals critically needed to acquire new knowledge about the deleterious health effects of environmental agents and to implement recent environmental legislation such as the Toxic Substances Control Act.

A summary of each of the Institutional training grants supported at the time of this writing appears in the report.

In October 1977, the Academic Investigator Award-Toxicology was announced in the NIH Guide for Grants and Contracts. A copy of the announcement was also mailed to university departments with current research and training activities in toxicology. The objective of this award is to retrain research scientists to pursue new careers in toxicology. Thus, the program calls for a major commitment, on the applicant's part, to the field. The initial response to the announcement was six applications. Four awards have been made at a total cost of \$160 thousand. Applications continue to be solicited for the program and it is anticipated that these awards will continue at a rate of five to ten per year.

Environmental Health Sciences (EHS) Center Programs

Environmental Health Sciences Centers, which are University-based, comprise an essential national resource and are an integral part of the strategy of NIEHS in serving the national needs in environmental health research. These Centers perform high quality, multidisciplinary research in areas of high relevance to the mission of NIEHS and serve to attract and train outstanding students to meet future research needs of the Nation. During FY-1978, the NIEHS supported eight EHS Centers with funds totaling \$8.7 million.

Because of the importance of the EHS Centers in meeting National needs, communication between the Centers, the Institute Director and his Staff, and the National Advisory Environmental Health Sciences Council (NAEHSC) is maintained at an effective level through biannual meetings of the Center Directors, frequent visits and telephone contacts by Extramural Staff. Members of the NAEHSC Subcommittee for Environmental Health Sciences Centers frequently accompany staff on oversight visits, as well as attending Center Directors' Meetings. The Subcommittee provides liaison between the Institute, the Advisory Council, and the Center Directors by keeping abreast and discussing research problems and accomplishments, new environmental concerns, and program directions.

The seventeenth meeting of Center Directors was held October 17-18, 1977 at the University of Cincinnati Center for the Study of the Human Environment, Kettering Laboratory, Cincinnati, Ohio, with Dr. Raymond Suskind as host. The first day of the meeting was given over to presentation of highlights of the program associated with the Center at the Kettering Laboratory. In the evening, Dr. Eula Bingham, Assistant Secretary for Occupational Safety and Health Administration, Department of Labor, addressed the group on the topic "A Newly Formed Bureaucrat's Views of Health and Safety." On the second day, the Directors of the other Environmental Health Sciences Centers gave 40-minute presentations of highlights of their research and training programs.

The eighteenth meeting of the Center Directors was held June 19-20, 1978 at the National Institute of Environmental Health Sciences in Research Triangle Park, North Carolina, with Dr. David P. Rall, Director, as host. After a report by Dr. Rall on the activities and new developments at the Institute, the morning of June 19 was given over to presentations of overviews and future plans of the new Laboratories at the Institute by the laboratory or branch chiefs. Beginning in the afternoon and continuing into the following morning, each of the program project directors presented 20-minute overviews of their programs. The remainder of the day was utilized by the Center Directors for highlights of research being conducted at each of the Centers. At the conclusion of the first day's program, guests were given a bus tour of the construction site of the new NIEHS facilities.

In July of 1977, the Marine and Freshwater Biomedical Core Centers Program of the Institute was announced in the NIH Guide for Grants and Contracts. Many inquiries and some two dozen letters of intent were received. Ten applications were able to meet the November 1, 1977 receipt date. The applications were reviewed by the initial review groups in the Spring of 1978 and by the NAEHS Council in May. Seven of the applications were recommended for approval and three were awarded sufficiently high priority scores to warrant funding. Thus, this Program was implemented during FY-1978 in accordance with previously set goals. A few additional letters of intent and applications are being received, so that additional applications may be funded in future years under this Program.

RESEARCH MANPOWER DEVELOPMENT SECTION

Training Programs

The NIEHS Extramural Training Programs support pre- and postdoctoral research training in the fields of environmental toxicology, environmental pathology, environmental epidemiology and biostatistics and environmental mutagenesis. Each of these programs has as its goal to provide appropriately trained manpower for careers in research pursuant to the missions of the Institute. Program descriptions and summaries are provided in the following text.

Environmental Toxicology

Training in environmental toxicology is focused on developing individuals for research careers pursuing an understanding of the pharmacological principles which determine the mechanism, site, and severity of damage to tissues by environmental agents. Research training focuses upon the processes by which environmental agents affect biological systems and by which biological systems influence the action and fate of these agents (metabolism, tissue distribution, etc.). Approximately 65 percent of our training support dollars are spent in the area of environmental toxicology.

To better define "environmental toxicology" let us divide working toxicologists into the following classes:

1. Clinical Toxicologists who deal with acute intoxication by poisons: These individuals determine causes of intoxication (which chemical?) and prescribe methods of treatment. They are usually associated with hospitals or poison control centers.
2. Experimental Toxicologists who are involved in the demonstration of safety or hazard of exposure to chemical or physical agents such as environmental chemicals or drugs: These individuals do testing on experimental models. They design protocols and analyze results. Their work usually culminates with a "safety evaluation" report including protocol descriptions and the data obtained in support of a conclusion on hazard level resulting from exposure to the particular agent.
3. Predictive Toxicologists who speculate on the hazard to man from exposure to chemical or physical agents: These individuals attempt to obtain knowledge about the pharmacodynamics and pharmacokinetics, mechanisms of action, dose/response relationships, comparative metabolism, etc. of the particular chemical involved. They do research on experimental models in an attempt to extrapolate this knowledge to better assess the hazards to man of exposure to the chemical.

4. Forensic Toxicologists who determine cause/effect relationships between poisons and pathologies: These individuals usually work with law enforcement agencies.

The NIEHS supports the training of pre- and postdoctoral students in the second and third classes of toxicologists, focusing on those individuals who wish to study the effects of environmental agents.

There are generally two schools of thought regarding how individuals should be trained for research careers in environmental toxicology. The philosophy of the first school is that a student must initially be trained as a scientist in a classical discipline and then receive additional training and experience to specialize in toxicology. This has been the general pathway by which today's established toxicologists have moved into the field. Individuals in this school argue that toxicologists must first be trained as good basic scientists so that the scientific method can be applied in well-defined experiments to determine the nature of the toxicity at the biochemical, physiological cellular or tissue level.

The philosophy of the second school is that toxicology is a discipline unto itself which draws skills and backgrounds from various facets of the basic sciences. Therefore, a toxicologist should be trained not only by taking courses in toxicology and in special topics which emphasize the interaction of environmental chemicals with the biochemical systems, organelles, macromolecules, or the tissue component.

The NIEHS Extramural Training Program in toxicology supports institutions training individuals under both these philosophies. Therefore, our award portfolio is comprised of grantees in a variety of departments such as biochemistry, physiology, nutrition and food sciences, etc., in addition to Departments of Toxicology.

It is becoming quite apparent that there are needs for toxicological specialists to engage in two types of activities. The immediate need is for toxicologists to perform safety evaluations to provide data required by the regulatory agencies upon which societal decisions can be made as to whether or not the general population should be exposed to a certain material. These are the experimental toxicologists referred to above. Likewise, there is an equal demand for individuals who will advance the state-of-the-art of the science of toxicology by furthering our understanding of toxicological mechanisms of action by developing new testing methodologies and by producing new knowledge about the effects of chemicals on organisms. This new knowledge will then allow safety evaluation procedures to become more precise and more capable of predicting the true hazards of these agents in the real world. Figure 1 shows how the training of toxicologists may differ beginning at the postdoctoral level depending on the choice of career pathway.

TRAINING OF RESEARCH TOXICOLOGISTS

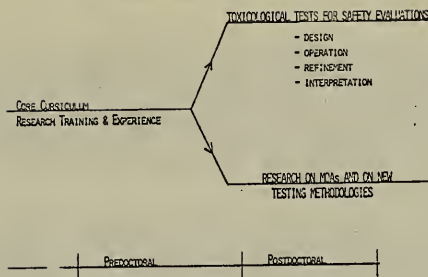


Figure 1

The demand for individuals completing their training in both these specialties of toxicological sciences is rapidly expanding. The demand for individuals capable of performing research in safety evaluation comes from all sectors of the economy. At least two recent surveys on the capacities of safety evaluation laboratories have concluded that the demand for such services in the next few years will far exceed the capacity of these laboratories to absorb the work. The limiting factor is the availability of trained individuals to design, conduct and interpret the testing protocols.

The demand for research toxicologists also comes from academia, government and private research laboratories and, in the long run, it is the activities of this group of people which will bring us out of the quagmire of uncertainty regarding how to do proper safety evaluation.

Grants for institutional support of new and extant training programs in environmental toxicology made during the past year are described below:

The Massachusetts Institute of Technology, Department of Nutrition and Food Science has a training grant to support pre- and postdoctoral students for training in biochemical, chemical and biological approaches concerned with problems relating to the public health impact of environmental chemicals. Specific thrusts are the identification of toxic, carcinogenic and mutagenic chemicals in food and water; the development of chemical and biological methods for their detection and quantification; the development of model systems for the study of their formation; the characterization of their acute and chronic toxicity, carcinogenicity and mutagenicity; the study of their metabolic activation and inactivation and attempts to elucidate their mechanisms of action.

The University of Rochester, School of Medicine and Dentistry, Department of Pharmacology and Toxicology has an award to provide pre- and postdoctoral training in environmental toxicology in a broad range or major problems such as behavioral, biochemical, developmental, and inhalation toxicology. Trainees select preceptors and thesis topics from the faculty of the Environmental Health Sciences Center at that Institution.

The training program at Vanderbilt University prepares pre- and postdoctoral trainees for careers in toxicology by emphasizing a chemical and metabolic approach to the structure, mode of action, and the mechanism of action or deactivation of a variety of chemicals found in the environment. Training emphasizes studies on metals, industrial toxicants, pesticides, mycotoxins, and other naturally occurring toxicants.

A grant to the Pulmonary Medicine Division of the Duke University Medical center supports the training of Ph.D. and postdoctoral scientists in environmental problems of the human lung. The focus of this training grant is on the defense mechanisms of the lung after exposure to environmental agents such as silicon dusts and pyrolyzed hydrocarbon particulates. The program is multidisciplinary utilizing faculty and facilities from several university departments.

The research training for postdoctoral and advanced predoctoral students at the Medical College of Wisconsin in environmental toxicology focuses on investigations of basic mechanisms of toxicant action. The program provides a mix between applied toxicology and basic toxicological principles with didactic training in biochemistry, physiology, pharmacology and toxicology. Research activities emphasize environmental toxicology, aquatic toxicology, human toxicology, chronic toxicology and systemic toxicology.

A training program at the University of Mississippi Medical Center in the Department of Pharmacology and Toxicology provides training in modern toxicological sciences, basic medical sciences, and pharmacological, pathological, and biochemical considerations which are related to the evaluation of the safety of toxic materials. The program is oriented toward a metabolic approach to the evaluation of the toxicity of chemicals which are found in the environment. Examples of such chemicals are food additives industrial toxicants, agricultural chemicals, air and water pollutants, and mycotoxins. Trainees receive instruction in all phases of toxicology including enzymology, isotope techniques, isolation and identification of metabolic products, and problems related to the comparative evaluation of animal species and man.

A multidisciplinary training program in toxicology is supported at North Carolina State University in the Department of Entomology. Trainees select from emphasis areas such as biochemistry, entomology, botany and zoology. Trainees receive instruction and research guidance from faculty members in these departments as well as from interactions with appropriate individuals in the laboratories of the NIEHS, the Environmental Protection Agency (EPA) and several private laboratories in the geographic area of this Institution.

A predoctoral training program at the Children's Hospital Research Foundation in Cincinnati provides trainees with a rigorous foundation for research careers in teratology. Training in molecular teratology is carried out through the interdisciplinary graduate program in developmental biology at the University of Cincinnati, which awards the Ph.D. degree. Students

approach the subject of teratology as an abnormal manifestation of development. The molecular aspects of this subject are emphasized and the mechanism by which environmental agents cause molecular perturbations in development and lead to physiologic, morphologic and functional deficits is studied. The role of pharmacodynamics and biotransformation of environmental agents is stressed.

The Department of Entomology at Cornell University has a training grant to provide predoctoral and postdoctoral training in several disciplines falling within the field of environmental toxicology. Primary focus is on the molecular interactions of toxic materials such as drugs, pesticides, industrial pollutants, and natural products with living organisms and the environment. Specific thrusts are in the areas of neuropharmacology, mechanisms of action of toxicants, metabolic degradation of environmental agents and assessment of toxicological hazard to man from these materials.

A grant emphasizing predoctoral training to the Department of Environmental Toxicology at the University of California, Davis stresses the chemical aspects of the science. Physiological and toxicological consequences of low-level exposure to toxicants are determined by the use of biochemical or behavioral endpoints. Structure-activity relationships to allow prediction of physiological distribution and of likely molecular interactions are sought. Metabolic differences between species are studied to allow better prediction of hazard to man through the use of experimental laboratory models for the assessment. Finally, natural toxicants in the human food supply are identified and risk assessments made.

The primary purpose of the training program at New York University is to train individuals for research careers in inhalation toxicology, including data evaluation and interpretation. Students learn to plan, conduct and interpret inhalation and other toxicological studies appropriate to the environmental health issue in question. Courses in the basic medical sciences, environmental health sciences, inhalation chamber technology, and the physical and physiological behavior of gases and aerosols are provided in addition to relevant research experience.

An award to the Johns Hopkins University School of Hygiene and Public Health, Department of Environmental Health Sciences supports pre- and postdoctoral students learning to evaluate and predict the effects of environmental pollutants on biological systems. Faculty from a number of biological specialties including pharmacology, physiology, biochemistry, biophysics, immunology, genetics and cellular and molecular biology participate in a program focused on learning the basic interactions of environmental chemicals with biological systems. Specific research interests of the staff include hepatic and pulmonary toxicology, neuro- and behavioral toxicology, inhalation toxicology, pharmacokinetics, developmental toxicology, mutagenesis, carcinogenesis, and the influence of genetic abnormalities on the response to environmental chemicals.

A Ph.D. program in environmental toxicology in the Department of Environmental Health at the University of Cincinnati College of Medicine consists of an

intensive core curriculum followed by specialization in environmental carcinogenesis, mutagenesis, or teratogenesis, or neurobehavioral toxicology.

A training program in biophysics at the Roswell Park Memorial Institute trains pre- and postdoctoral students to study the effects of environmental agents on biological systems at the molecular level. Specifically, changes in biomolecular structures caused by agents such as chemical carcinogens, ionizing radiation, ultraviolet radiation, and repair inhibitors are stressed.

A grant to the Institute for Environmental Studies at the University of Illinois supports pre- and postdoctoral trainees in the areas of pharmacokinetics of toxic substances, detection and characterization of micro-pollutants, comparative toxicology, study of biocides for selective toxicology, and studies of the movement of toxic chemicals through the environment.

An award to Duke University Medical Center, Department of Physiology and Pharmacology trains postdoctoral students in the biochemical, physiological and pharmacological aspects of environmental toxicology. Specific areas of emphasis are enzymology, cell biophysics and integrative physiology.

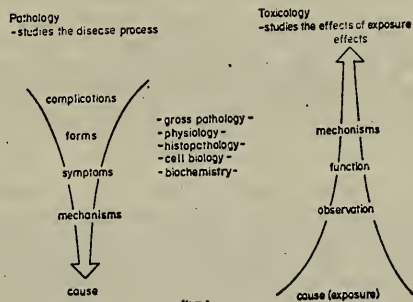
The Center for Environmental Toxicology at the University of Wisconsin is supported to provide postdoctoral training in general environmental toxicology. Examples of special research training areas at this institution are mechanisms of action of chlorinated hydrocarbons on mammalian tissues and on post-defense mechanisms, identification and metabolism of mycotoxins, carcinogenesis determination, solvent toxicity, pesticide toxicity, and the nature, persistence and toxicity of organic chemical byproducts.

In addition to these institutional awards, 27 individual postdoctoral fellowships in toxicological sciences are being supported. The majority of these are giving training to individuals pursuing careers in research in toxicology with emphasis on materials such as heavy metals and ambient organic chemicals. Four of these awards are for training in behavioral toxicology and two are for research training in ecotoxicology.

Environmental Pathology

Trainees in this area focus their research on factors involved in chemical (as opposed to infectious disease) pathology. Typically, trainees hold professional or academic degrees which qualify them for advanced training in gross and histopathological research dealing with the structural and functional alterations of tissues exposed to environmental chemicals. Usually training is focused on morphology, but training in the biochemical basis of environmental disease pathogenesis may also be included in these programs and the tools of the toxicologist can also be used to elucidate the mechanistic bases of environmental diseases.

Figure 2 illustrates the difference in thrusts between environmental pathology and environmental toxicology training.



The objective of the pathologist is to determine the cause of the observed lesion, whereas the objective of the toxicologist is to assess the effects of exposure to a known agent. Nevertheless, the tools used by both disciplines in the various facets of investigation may be identical. Therefore, graduates of the environmental pathology training programs can become members of research teams involved in chemical risk evaluation utilizing laboratory animals as experimental models. Although these graduates are capable of histopathological evaluation, their backgrounds allow them to become more active members of research teams involved in chemical risk evaluation or to become independent investigators.

Four institutional awards for training in environmental pathology, described below, comprise the current program.

The environmental pathology training program at the University of North Carolina School of Medicine provides postdoctoral training in experimental environmental pathology and human comparative pathology of diseases and lesions produced by chemical and physical contaminants in the environment. Postdoctoral trainees work in an apprenticeship relationship with senior scientists on research involving the pathogenic effects of environmental contaminants such as heavy metals, carcinogens, and mutagens, particularly as they relate to DNA replication and repair, abnormalities of hemostasis, and cell and tissue ultrastructural effects.

The environmental pathology program at the School of Veterinary Medicine at the University of California, Davis allows postdoctoral trainees 12-18 months to take advanced courses appropriate to pathology and environmental toxicology and thereby to become familiar with environmental health problems and research needs. The training emphasizes observation of morphological abnormalities resulting from exposure to environmental agents, and interpretation of these abnormalities in a manner which generates new knowledge about their effects. Neuropathology, pulmonary pathology, teratology, and developmental immunology are stressed in this program.

The Department of Pathology at the University of Washington School of Medicine likewise trains in the morphological manifestations of exposures to environmental agents. A broadly-based training faculty in the related fields of toxicology, pathobiology, teratology and behavioral toxicology participate in training individuals to have strong capacities to investigate the cellular and sub-cellular effects of exposure to contaminants on the nervous, respiratory, renal and gastro-intestinal systems. Likewise, the effects of congenital exposures to environmental agents on brain structure and behavior as well as pathological studies are pursued.

The Washington University (St. Louis) School of Medicine Department of Pathology received support for training pre- and postdoctoral students in the areas of environmental carcinogenesis, the effects of environmental agents on the developing nervous system, the relationship between air pollution and chronic diseases such as emphysema, hypertension and arteriosclerosis, and on basic mechanisms in cell injury and death induced by environmental agents.

The Department of Pathology at the University of Wisconsin Medical School received support for a multidisciplinary training program emphasizing the pathological changes which arise in mammalian tissues from exposure to environmental toxicants, such as halogenated hydrocarbons and heavy metals. A wide range of research experience is available to these trainees including ultrastructural morphology of affected cells and their functional states. This training program draws heavily from the resources of the primate center at that Institution.

Environmental Epidemiology and Biostatistics

Trainees in environmental epidemiology are taught to utilize statistical and mathematical tools to assist in the identification of environmental diseases in human populations. Training stresses non-infectious disease epidemiology with emphasis on the identification of causes of environmental diseases. The major difference between training in environmental epidemiology and infectious disease epidemiology is that human populations are studied with respect to the effects of established exposures to environmental agents, whereas epidemiological studies of infectious diseases usually begin with a pathological observation.

Biostatistics trainees learn and apply mathematical and statistical tools assisting in environmental health scientists in experimental design and interpretation. Mathematical modeling for human risk assessment based on laboratory experiments is studied. Methodologies for extrapolating the results of high dose exposures to environmental agents to predicted effects of real-world low dose exposures are also developed. Upon completion of their training, these individuals undertake research activities supportive of the development of laws and standards governing human exposures to environmental agents.

In addition to one postdoctoral fellowship in epidemiology and one in biostatistics, current support for institutional training was as follows:

A grant to Harvard University School of Public Health, Department of Epidemiology trains pre- and postdoctoral students for research investigations on the effects of environmental chemicals on humans. A combination of courses in environmental health, biostatistics, epidemiology, toxicology and occupational health precedes research activities in this field. Postdoctoral students (usually post-M.D.) also receive a Master of Science in Epidemiology from this program.

The University of North Carolina (UNC) School of Public Health Department of Epidemiology likewise trains students in the principles and perspectives of epidemiology and biostatistics as well as environmental sciences and human biology to develop a capability to initiate epidemiological inquiries to test hypotheses on the biological effects of environmental agents. Subsequent to didactic training, a research problem is selected drawing on the resources of the University, the Environmental Protection Agency, the National Institute of Environmental Health Sciences and the Occupational Health Studies group at the UNC.

A program at the University of North Carolina in biostatistics emphasizes the mathematical aspects of research in environmental health studies and the provision of statistical support to workers in the field. Trainees participate in research problems on measurement of individual environmental hazards, interactions of several hazards, measurement of doses and responses in human populations and estimation of dose-response relationships.

A training grant to the Department of Statistics at the Florida State University supports pre- and postdoctoral trainees in statistics to assist in research involving environmental measurements. Biometrics and statistics are utilized on multidisciplinary problems, monitoring pollutants or pathogenic substances in the environment.

Environmental Mutagenesis

Trainees in this area are taught to apply the basic principles of genetics to applied studies aimed at assessing the potential genetic hazard to man of environmental chemicals. Training emphasizes: (1) the understanding of chemical factors which predict a compound's ability to alter the genetic makeup of man, (2) the development of reliable test systems with unequivocal quantitative relevance to man for detection and quantification of mutations in germinal cells, and (3) the elucidation of molecular and cellular mechanisms in mutagenesis.

This is the smallest area of NIEHS training support and is comprised of minor parts of several of the aforementioned institutional awards in environmental toxicology and two postdoctoral fellowships.

HIGHLIGHTS - SPECIAL PROGRAMS

In this report we are shifting to new categorical descriptors which will be used in the future, i.e., (1) Prediction, detection, and assessment of environmentally-caused diseases and disorders, and (2) Mechanisms of environmental diseases and disorders.

I. PREDICTION, DETECTION, AND ASSESSMENT

FIELD, EPIDEMIOLOGICAL, AND CLINICAL STUDIES

The substances dealt with in field, epidemiological, and chemical studies include essentially all types of environmental pollutants in all media (air, water, food). Thus any categorization of substances is essentially a matter of convenience. For this report, studies are reviewed in terms of (1) general air pollutants, (2) work-place related pollutants, and (3) therapeutic and cosmetic agents (self-administered substances).

1. General Air Pollutants. Centers and program projects include numerous studies on health effects of gaseous and particulate pollutants which are ubiquitous and are encountered by everyone to some extent. These include gases such as SO_2 , NO_2 , O_3 , CO , CO_2 , and certain hydrocarbons; and particulates such as SO_4^{2-} , silicates, smoke, fly ash, aerosols, soots, and a variety of organic particulates.

An important study of adults and school children exposed to SO_2 , NO_2 , O_3 , SO_4^{2-} , and suspended particulates is being conducted in six cities of the United States. Pollution varies substantially from one of the cities to another, generally representing the more heavily and least polluted cities of the United States. Data are being obtained on x-ray and pulmonary function abnormalities, general health status, age, sex, body features, race, occupation, ethnicity, and any other factors that may have a bearing upon health response to the pollutants. Preliminary data show higher than normal non-specific lung disease in two of the more heavily polluted cities, but the data are not yet adequate for rigorous conclusions.

Similar tests are being conducted on other populations, including toll booth operators, firefighters, and residents at various distances from various kinds of industrial installations. A few studies are being conducted on special groups, such as firefighters, who may be exposed to HCl and HCN , as well as the usual gaseous pollutants. Preliminary data in one such group under study since 1970 show twice the expected rate of lung function deficits, particularly in smokers, and some differences between ethnic groups.

2. Work-place Related Air Pollutants. Many studies are in progress concerning health effects of pollutants associated with the work-place. Pulmonary function in polyurethane foam workers has been shown to be lowered both by acute and by chronic exposure to 0.002 - 0.013 ppm

toluenediisocyanate. Workers in crushed velvet plants have been shown to develop a peculiar disease called acute polymer fume fever, characterized by coughing, fever, chills, aching, and weakness. This has been traced to pyrolysis products of fluorocarbon polymers used to make crushed velvet. The fever occurs only in smokers and the pyrolysis products are formed from dusts on the cigarettes of the smokers.

A somewhat different syndrome has been traced to another fluorocarbon, Freon 22. It was found that cardiac arrhythmia in pathology residents in a Massachusetts hospital was 3.6 X that in radiologists. The arrhythmia was traced to use of Freon 22 in the preparation of frozen tissue sections in the pathology laboratory, which was rather poorly ventilated. The long-term effects of Freon on heart function are unknown.

Excess cancer has been shown to be associated with a number of occupations. Compared to the general population, rubber workers have been found to have about twice the incidence of stomach cancer of the normal population, 1.4 X the cancer of the large intestine, 1.2 X the bladder cancer, and 1.3 X the incidence of leukemia. There have been strong indications of increased cancer among styrene and polystyrene plant workers, vinyl chloride polymerization workers, bis(chloromethyl)ether exposed workers, and workers exposed to benzo(a)pyrene and associated substances in roofing and waterproofing trades employing hot pitch and asphalt.

A large number of effects other than cancer have also been associated with worker exposure to volatile substances. Increased conjunctivitis and localized erythema have been found to occur in roofers exposed to pitch, asphalt fumes, roof dust, etc. Depressed pulmonary function, aggravated by smoking, has been documented in welders. In addition to already well-known effects of PCBs (polychlorinated biphenyls), evidence of effects on immunocompetence, particularly of T cells, has been obtained. PBBs (polybrominated biphenyls) apparently have similar effects on lymphocytes, but in addition may cause neurologic disorders and other ill-defined adverse health effects.

Many other studies are in the preliminary stage or are well underway from which results are not yet available. They concern numerous volatile and industrial substances, such as: oxidant air pollutants, anesthetics, paint solvents, degreasers, dry cleaning fluids, trichlorophenol, dioxins, aniline dyes, nitrosamines, and various wood preservatives. Essentially all pertinent health parameters are being observed, including especially: x-rays, pulmonary function, cigarette smoking, ethnicity, sex, age, present and antecedent health status, blood chemistry, urinalysis, etc., in clinical and prospective studies. When the records permit, these kinds of data are also being obtained in retrospective studies. However, more often these studies are limited to analysis of death certificates, hospital records, social security records, etc.

3. Therapeutic/Cosmetic Agents. A few studies are being conducted on cosmetic and therapeutic agents, though such agents do not represent a major focus of NIEHS. There is much interest and considerable controversy regarding the carcinogenicity of arsenicals. An opportunity to examine the possible long-term effects of one compound, Marpharsen, has presented itself in a population of 1300 syphilis patients administered 0.1 - 3.0 g of the arsenical from 1940-1947. Mortality by cause, particularly cancer, is being determined from death certificates and social security administration records. No definitive results are available as yet.

Hair dyes (aromatic amines) are being evaluated as etiologic factors in some 200 breast cancer patients. Analysis of data will take into account the duration and frequency of hair dye usage, but the study is not yet complete.

In a much more extensive study, some one million patients treated with isoniazid for tuberculosis between 1931 and 1960 are under observation for increased cancer (type and site). Analysis of the data is in progress but definitive results are not yet available.

Several studies are focusing on groups exposed to x-rays in the treatment of various problems. In a case-control study of 1300 children exposed prenatally to x-ray, it was found that the incidence of leukemia was 1.5 X normal. There was no increase in solid tumors. Similar studies of children treated with x-ray in the 1940-1950s for tinea capitis showed increased cancer of the thyroid, increased leukemia, and excessive hair loss and opacities of the lens.

Several studies concern the possible role of x-ray treatments in breast cancer. In a follow-up of women treated with x-ray for post-partum mastitis, there was found to be a 2.2 X risk of breast cancer, with a linear dose-response relationship after 15 years. In another group of women with x-ray exposure of the breasts in fluoroscopy of tuberculosis, the risk of cancer was about 2 X in women below age 29 but was not increased in women above that age at the time of fluoroscopy.

4. Particulate Air Pollutants. The health effects of particulate matter in the air is of major concern in many occupations and to the general population. Virtually all such pollutants are under study in efforts to characterize the particulate material, to devise ways of measuring the quantities to which individuals are exposed under various circumstances, and to identify threshold changes in target organs that signal future serious health problems.

Though several studies have been conducted on possible health effects of titanium oxide in paint, paper, etc., no problems have been noted with this substance. Lead oxide, on the other hand, has been a major health problem in children in the past. It remains a potential problem in some localities where old paint remains in and on old buildings. In general, epidemiological studies indicate that the lead blood levels in painters is declining in recent years, due presumably to removal of

PbO as an ingredient of paint. However, a potentially serious exposure may exist in secondary lead smelter workers, where clinical examinations indicate blood levels often in excess of 80 ug/dl.

By far the greatest public concern about lead relates to exposure in children. Discernable abnormalities in behavioral patterns of children have been found as sequela in lead encephalopathy cases. These are cases of extreme lead intoxication and at present a number of studies are focused on children with much lower levels of exposure. Clinical studies of the effects of subclinical lead burden (40 - 70 ug/dl) on intelligence, perceptual motor skills, behavior, and achievement involving 80 preschool children indicate that those with moderate lead burdens performed more poorly on general cognitive, verbal, and perceptual scales. These tests are being repeated. Other studies are also in progress to determine the relationship between time and amount of lead exposure to neuropsychological functioning.

Similar clinical and epidemiological studies are being conducted on mercury and its compounds. A number of populations with various sources of mercury are under study, including Iraqi mothers and their infants exposed to mercury through treated grain seed; women in the White Dog and Grassy Narrows Indian Reserves in Canada; Samoan and Korean fishermen whose diets consist of fish with considerable mercury contents; and mercury miners in Spain, who are exposed both to mercury in vapor form and to cinnebar dust. A number of these are ongoing studies for which data are incomplete, but in the case of the Iraqis exposed to mercury through treated wheat made into bread, considerable irreversible brain damage has been documented in surviving infants exposed in utero. The aim now is to establish the threshold prenatal exposures which produce discernible problems in the newborn and the developing child, and to determine the dose-response relationships between exposure and such effects as paresthesia, ataxia, deafness, dysarthria, and mortality.

Mineral and organic dusts represent serious problems in many occupations and often in the home environment. Studies of granite shed workers and talc millers and miners indicate that these individuals eventually suffer reduced ventilatory capacities. Studies of textile workers have identified many individuals with abnormal chest x-rays. Research is now in progress to clarify the mechanism of pulmonary response and pathogenesis on exposure to various kinds of organic particles.

Of all hazardous dusts, asbestos appears to be one of the more insidious and widespread. Exposure groups fall naturally into several categories. There is a large number of workers directly in contact with asbestos. Among these are asbestos miners, insulation factory workers, insulation installers, and asbestos cement workers. In studies of insulation workers between 1943-1962, it was found that about 20 percent of all deaths in the cohort were due to cancer and 7 percent to asbestosis. Of the cancers, 6 percent were mesotheliomas. Continuing studies of a larger group of insulation and asbestos workers suggest an increased incidence of cancer of the larynx, oropharynx, and kidney. No increases in leukemia, lymphoma, or cancer of the bladder or liver have been noted.

A number of studies are being conducted on people not involved directly in handling asbestos, but exposed through indirect contact. Such people include tilesetters, roofers, automobile brake repair workers, drywall installers, toll booth fare collectors, and those employed in many trades in shipyards. One case of mesothelioma has been reported in the cohort of toll collectors. Random cases of this cancer have been identified among brake repair and maintenance workers, but the major concern at the moment centers around shipyard workers. After 15 years, 45 percent of those examined had x-ray abnormalities (parenchymal and pleural). Based on past experience, it is predictable that a substantial number of these workers will develop cancer of the lung later in life.

Other studies involve the families and neighborhoods of asbestos workers. Wives and children of the workers are exposed to asbestos dust brought home on the workers' clothing. In some cases, entire neighborhoods are exposed by virtue of the presence of insulation or other kinds of factories from which asbestos dust emanates more or less continuously over many years. Studies of these populations are underway but conclusive data on risk are not yet available.

Extended clinical studies are being conducted also on various exposure groups. These involve hospital examinations for increases in colon cancer, particularly tumors with proximal distribution typical of those related to asbestos exposure. The studies include also effects of smoking, age, duration of exposure, time since exposure, etc., on pulmonary fibrosis. In addition, efforts are being made to work out routine detection methods for pleural plaques and for determining their significance in pleural mesothelioma.

Extensive quantitative evaluations of dust of various kinds in diverse locations are being made. These are designed to serve two purposes. (1) for correlation with disease in cohorts currently under study, and (2) for identification of possible risks. Among the places and products where measurements are being made are work environments, workers' homes, schools and other buildings, rock quarries (for road-bed stone), water supplies, and a number of consumer products, including talcs, parental drugs, spackling and patching compounds, talc-coated rice, etc.

5. Occupational/Geographic Disease Association Surveys. Since the survey by the National Cancer Institute showing distinct disease clusters associated with geographic locations throughout the country, attempts have been made to establish localized associations of diseases such as oat cell carcinoma, bladder cancer, prostate cancer and other diseases with geographic location and/or occupation. Transportation workers have been shown to have 2 X the normal incidence of oat cell cancer of the lung. There is a significant excess of bladder and/or prostate cancer among cigarette smokers, rubber workers, and leather workers, with an earlier than normal onset of disease. A very extensive study is being conducted to evaluate multiple factor interactions. This study involves about one million people from AFL-CIO and American Cancer

Society records concerning mortality, birth defects, miscarriages, and stillbirths, taking into account social habits, occupation, place of residence, medical history, medications, etc.

6. Supportive Research and Activities. A number of projects and activities in support of epidemiologic, field, and clinical studies also deserve mention. Efforts are being made to develop computer and/or mathematical models of the chemodynamics of pollutants, the kinetics of tumor formation, mortality trends, methods of extrapolation from high to low doses, etc. In addition, many projects are in progress to improve air sampling devices for gases and particulates, chemical analytical methodologies, and analytical electron microscopic techniques for particulates of environmental health significance.

II. DISEASE AND INJURY MECHANISMS

While the identification and immediate assessment of the health effects of pollutants must be accomplished through direct studies of the victims, the long-range potential health effects that may be manifested many years hence and perhaps in future generations require a different approach. Traditionally, this approach utilizes basic sciences as tools for development of a body of knowledge with which one can make useful predictions about health effects. The major portions of the resources of the Institute are devoted to studies of this nature.

1. Volatile Pollutants. Because SO_2 has been shown to be toxic at high dosage levels, it has been assumed to be toxic at or near levels in the air over large cities. Numerous studies are in progress on various aspects of SO_2 chemistry and toxicology. It has been shown that SO_2 undergoes rather rapid changes in the atmosphere, forming more highly oxidized species which combine with water vapor to form strong acids, which, in turn, may react with a wide variety of substances to form salts and/or become adsorbed on solid atmospheric particulates to become a part of the general atmospheric aerosol. Similar reactions are postulated and are under investigation with respect to the cation NH_4^+ in the respiratory tract. While the formation of NH_4^+ salts, which adsorb water to form hydrated sols, has been demonstrated, the significance, if any, of such species in the lungs is unknown. There is some evidence that SO_2 or its derivatives may lower the resistance of experimental animals to viral and bacterial infections. There appears to be good evidence that the substance(s) reacts with lung epithelium, causing sensory irritation. However, there is also evidence that such injury may lead to epithelial atrophy and subsequent repair resulting in epithelial cells more resistant than normal to toxic gases.

There is some evidence that SO_2 , benzo(a)pyrene, and particulates in combination will increase the risk of lung cancer and earlier onset of disease in experimental animals. This is explained in part at least by understanding some basic studies of the chemistry of the interactions

of SO_2 when it is absorbed into the blood stream. It has been shown that SO_2 reacts with blood thiols to form thiol-sulfonates. It may also be acted upon by a reductase which converts it to sulfite. In either of these forms it can react with cellular thiols, purines, and pyrimidines. In the case of the bases, at least three reactions have been demonstrated, i.e., deamination of cytosine, transamination of cytosine and saturation of uracil (formation of sulfone). These reactions are consistent with observed inhibition of t- and m-RNAs and protein synthesis and with mutagenic effects in certain organisms.

Studies are continuing on a second major class of air pollutants derived from combustion and/or the interaction of smog and sunlight, e.g., carbon monoxide, oxides of nitrogen, and ozone. These studies have been covered rather comprehensively in previous reports. The principal effects, interactions, etc. seem to have been fairly well defined as they pertain to adult rodents. However, some differences in susceptibility related to age of mice have been discovered which have unknown significance for man. It was found that neonates are resistant to O_3 and NO_2 until weaning. This sudden change in resistance coincident with weaning suggests an important relationship between diet and resistance and the basis of this relationship is under intensive study. It should be noted that previous studies have also indicated relationships between resistance to oxidant pollutants and dietary factors such as Vitamins A and E and zinc. There is no good evidence that the resistance level of the adult animal can be raised. However, the situation with regard to neonates is unknown. Moreover, there is no indication whether vitamins or some other factor(s) might be responsible for neonate resistance.

Recent studies have raised the awareness of occupationally-exposed individuals to the hazards of solvents, particularly benzene and styrene. Benzene depresses lymphocyte levels and induces aplastic anemia in mice at about 300 ppm in air. Histologically, hypoplasia in bone marrow is evident and exposed mice show a higher percentage of chromosome breaks and hypodiploidy than non-exposed animals.

Studies of a similar nature are being carried out on styrene. Styrene oxide, styrene glycol, and metabolites of styrene have been tested for mutagenicity in the Ames Salmonella test. Only styrene oxide was found to be active, indicating that styrene must be activated to be mutagenic. Clinical studies of styrene workers show significant effects, e.g., increase in polymorphonuclear leucocytes, low hemoglobin, obstruction of pulmonary airflow, acute conjunctivitis, and often a history of cough, wheezing, and shortness of breath.

Many halogenated compounds in extensive use in commerce are under study in various laboratories - compounds such as vinyl chloride, vinylidene chloride, trichloroethylene, allyl chloride, ethylene dichloride, epichlorohydrin, dimethylcarbamoyl chloride, etc. Interest in these compounds stems from the volume of their usage in chemical industries as intermediates or, in some cases, end products for use in various ways, and the relatively large proportion of such compounds that exhibit

some kind of biological effects. Many of these substances are carcinogenic, mutagenic, and, perhaps, teratogenic. Many others are directly toxic at relatively low doses to vital organs such as the liver.

Another class of compounds under study are polyaromatic hydrocarbons and polynuclear heterocyclics. Studies of benzo(a)pyrene are particularly numerous and intensive. Studies include seasonal aerometric surveillance and interactions with soot and other carbonaceous materials in the air and in cigarette smoke. Several studies deal with metabolism and activation of PAH species and with carcinogenic mechanisms.

Several studies are being conducted on the status of oil and other hydrocarbon mists and vapors in the atmosphere. Of particular interest is the photolysis of these materials and the potential and conditions for formation of carcinogens in the gas phase.

2. Suspended Particulates (Aerosols). The mechanisms of effects of aerosols and dusts of all sorts are under study in many laboratories. Basic studies on asbestos fibrogenesis and carcinogenesis range from electron microscopic studies at the subcellular level to those dealing with the relationships between surface properties of foreign particulates and reactions of living systems at the molecular level. Comparative studies are being conducted on different types of coal in an effort to identify the chemical and physical properties of coal dust associated with pneumoconiosis.

Similar studies are being carried out on cotton dust in an effort to identify chemotactic substances and to understand their roles and mechanisms in diseases such as proteinosis, fibrosis, and brown lung. Other studies deal with substances such as silica, talc, fiberglass and many others, in situations leading to exposure to single pollutants and to various gaseous and solid pollutant combinations.

3. Heavy Metals. The most extensively studied of the heavy metals is lead (Pb), both in the laboratory and in human populations. Its effects are many but those on the nervous system are generally believed to be the most serious. Pb may accumulate generally throughout the peripheral and central nervous systems. It appears to accumulate to higher levels in younger animals. For example, at the same level of administration, it was found to reach twice the level in the blood of ten-day-old rat pups as twenty-day-old pups. This was attributed to lower renal and biliary excretion in the younger animals. In addition to effects on nerve cells per se, effects have been demonstrated on certain hormones such as dopamine, noradrenaline and pituitary follicle stimulating hormone. In addition, effects have been demonstrated on heme synthesis and on kidney function.

Mercury is also being studied extensively. The studies focus on neurological and behavioral effects, teratological effects, interaction with other metals and with natural metabolites, and on factors, such as age and diet, which play a role in mercury toxicity. As with Pb,

efforts are being made to find new chelating agents useful in removal of Hg from the body and effective in preventing damage.

A number of studies deal with various aspects of cadmium toxicity. A principal concern in the past has been the ability of Cd to cause substantial elevation of blood pressure. However, more recent studies indicate other effects of Cd, particularly effects on the immune and nervous systems, which need further study.

Several radionuclides are under study, dealing primarily with distribution and retention in the body. Of particular interest are plutonium, radon, americium, and curium. Studies of curium, an alpha emitting actinide important in the nuclear fuel cycle, exceeds both Am and Pu production in nuclear power reactors. It is a bone-seeking substance with a retention of 200-300 days. The dose and retention time of several of these substances have been found to be modified by chelating agents, and studies evaluating such agents are continuing.

Other heavy metals and metalloid substances under study include selenium, arsenic, vanadium, molybdenum, copper, nickel, and combinations of these with each other and with other substances. Some of these and/or their compounds are thought to be carcinogenic; others are teratogenic or general toxicants. All studies are aimed at understanding toxicity mechanisms and potential hazards to specific workers and the general population.

4. Industrial Chemicals and Byproducts. In terms of volume, industrial chemicals are the foreign compounds to which the total population is exposed at the largest doses. Generally, they are well-screened and the bulk of the more toxic ones well-contained. On occasion, however, accidental toxic exposure of humans occurs and, often, unsuspected effects of commercial products are discovered.

One of the more problematic classes of compounds is the halogenated organics. These include such substances as the polychlorinated and polybrominated biphenyls, many antibacterial substances, pesticides, etc. A great deal of effort is applied to identifying the health effects of such compounds and the mechanisms of effects. For example, the effects of PCBs and PBBs on reproduction and the immune system is under intensive study, both in laboratory animals and in exposed human populations. Studies are underway to assess the potential hazards of antibacterial agents used in cleansers and soaps. Special emphasis is placed on studies of the nervous system which has been reported to be damaged by these substances. Studies are continuing on dioxins and dibenzofurans and on numerous halogenated phenolics, where dioxins occur as important and dangerous contaminants.

Other major classes of compounds are under study from the standpoint of general toxicity and/or effects on the nervous system, genome, reproduction, or on specific organs or functions. These include many amines, phthalates, and nitroso compounds.

5. Food Additives and Adulterants. Though many substances used in foods, cosmetics and drug preparations are separated for discussion, they may not differ basically from many other compounds entering the human system through other media. Thus, in general, the same approaches, rationale, and test systems are being used in the study of food additives and adulterants as are used for substances in other media.

Though there is a large number of substances in foods which can be treated as unremarkable toxic substances, there are substances which are likely to be found almost exclusively in foodstuff; i.e., phytotoxins and mycotoxins. Certain of these, such as aflatoxins, are extremely potent carcinogens. Others, such as penitrem A, are powerful neurotoxins; still others affect the kidneys, lungs, and/or other vital organs. Efforts are being made to identify all such naturally occurring substances of potential importance in foods and to determine what organisms synthesize them, their persistence and occurrence, and the basic aspects of pharmacology and pathogenesis associated with their ingestion.

6. Pesticides. Pesticides represent an extremely broad spectrum of toxic substances, often with little in common chemically. Thus, relating structure of these compounds to biological activity is a major activity which has been in progress for many years and is still far from complete. Studies are continuing in all major agricultural facilities all over the world to understand better the important aspects of pesticides, such as distribution, persistence, degradation products and mechanisms, metabolism by various species, toxicity among species, and the role of diet, species, age, and other factors in species susceptibility and resistance to toxicity. NIEHS supports work in all of these areas.
7. Physical Factors. Though the major effort in NIEHS programs concerns chemical toxicants, a considerable number of studies are concerned with physical factors, including UV and visible radiation, microwaves, lasers, noise, cold, heat and olfactory irritants. Studies of UV and light are concerned specifically with matters such as photochemistry, phototoxicity, photocarcinogenicity and phototherapy. Efforts are being made to improve the efficiency, precision, and specificity of instruments for photobiological studies, and the efficiency and specificity of light sources for treatment of "bronze" babies (hyperbilirubinemia). Nondermatological, nonvisual effects on the pineal gland, and other endocrine systems are also being studied.
8. Mutagenesis and Carcinogenesis. Studies of mutagenesis and carcinogenesis are primarily in two areas: (a) development and validation of test systems and (b) mutagenic and carcinogenic mechanisms. Test systems include the Ames *Salmonella typhimurium* test; *Drosophila* tester stocks; eukaryotic organisms for recessive mutations, nondisjunctions, crossing over, and translocations; human lymphoid cell cultures; animal lymphoid cell cultures; Chinese hamster ovary cells; biochemical tests; and tests on umbilical cord blood. The aim in all of these is to develop test systems which will detect essentially all types of mutations, permit translation of test results to man, and provide understanding of how mutations occur and how they might be prevented.

Mutagenic and carcinogenic mechanism studies include efforts to elucidate cell transformation, the basic phenomena in co-carcinogenesis and promotion, DNA repair, DNA structure and repair in relation to function, and quantitative aspects of mutational events and carcinogenesis.

The studies cover a wide range of compounds, including particularly vinyl chloride and related compounds, polyhalogenated biphenyls, aminonaphthalene and other amines, benzo(a)pyrene and many other substances produced naturally by fungi.

III. DEFENSE AND TOLERANCE MECHANISMS

Many studies are concerned directly with or involve some aspects of body defense and tolerance mechanisms. While defense systems may seem less important in chemical injury than in infectious diseases, there is already strong evidence that defense systems are vital to survival in a chemical world.

1. Mechanical Clearance, Exclusion, and Related Processes. A number of systems function to clear and/or exclude foreign materials from the body. Among these are airway cilia. Model studies of the mechanics of fluid movement and transport of debris are underway in this area. Numerous studies are concerned with macrophages. Of particular interest are the origin and proliferation of macrophages, the effects of gases and particulates on phagocytosis and macrophage regeneration, and the possible role of macrophage enzymes in the destruction of airway epithelium.

Airway epithelium per se is also under intensive study as the source of surfactant. Cellular regeneration and resistance of regenerated lining cells to noxious gases are of particular interest, as is the role of age, sex, and species as determinants of injury.

In addition to the active processes that tend to diminish the impact of toxic substances are a number of passive systems, e.g., the air-blood barrier of the lungs, the placental barrier, the blood-brain barrier, and, of course, the intestinal wall and the skin. These barriers are not only quite effective against solid particles but often are quite impermeable to certain gases and chemicals in solution on a selective basis.

2. Storage and Immobilization of Toxic Substances. Another passive protective mechanism involves storage (binding) of toxic substances. Adipose tissue serves as a sink for many fat-soluble, water-insoluble substances such as halogenated organic compounds. However, studies over the past several years have shown that immobilization of the compounds may be temporary. They may be gradually mobilized, often

showing up in mother's milk and being transferred to offspring. Many substances, particularly heavy metals and radionuclides, may be bound in bond and/or hair, where they are released extremely slowly. Certain toxic substances, notably lead, may remain at elevated levels in blood and other tissues for long periods after termination of exposure. The binding sites and reasons for the slow clearance in such cases is unknown.

3. Metabolism and Excretion. The most active defense mechanisms of the body are those involving enzymatic degradation of foreign and endogenous chemicals followed by excretion. The most important enzyme systems, all of which are presently under intensive study, are conjugases, dehydrochlorinases, epoxidases, and arylhydrocarbon hydroxylases, mixed function oxidases, and glutathione peroxidases, reductases, and S-transferases. One or more of these enzymes act upon almost every organic chemical known to enter the body. They serve generally to yield derivatives which are less toxic and easily excreted. However, in a few cases, such as with parathion and some related phosphates, the first products of metabolism may be more toxic than the parent compound. Given time, however, almost all compounds will be degraded and excreted if the systems are not overwhelmed by the toxic agents.

Some substances, particularly heavy metals and a few highly halogenated organic compounds are metabolized very slowly if at all. In the case of the metals, the body produces a rather unique protein called metallothionein which has the capacity to bind and detoxify metals. These proteins are under intensive study.

In the case of unmetabolized halo-organics, the body is able to eliminate them slowly without metabolism by excretion in the bile. This process can be enhanced if the compounds are complexed with certain substances. One useful substance, cholestymin, has been found and others are presently being sought.

RESEARCH HIGHLIGHTS

Regular Research Grants Program

The regular research grants program encompasses the Research Project Grants, the Research Career Development Award Program, and the Young Environmental Scientist Health Research Grants.

The Young Environmental Scientist Health Research Grants Program was initiated this past year, during which 19 grants were awarded. This program was designed to encourage investigators to develop an interest in problems in the environmental health sciences, and to provide support for their initial independent research efforts. It is gratifying that the diversity of interests exemplified by these young investigators, is compatible with the broad mission responsibilities of the NIEHS.

A valuable means of encouraging and developing research talent and leaders in the field of environmental health sciences is through support of the Research Career Development Award Program (RCDA). Currently, the NIEHS Extramural Program sponsors 12 research investigators under this program. The great diversity in the training and the variety of academic departments in which these investigators are working, and the disciplines represented, are characteristic of the field of environmental health sciences. Included in this group of 12 investigators are those holding Ph.D. and M.D. degrees, and such departmental affiliations as Community Medicine, Nutrition and Food Sciences, Oncology, Pharmacology, Human Biology, Otolaryngology, and Biostatistics. The research areas include: noise, pharmacology, toxicology, biostatistics, and carcinogenesis. The high caliber of the participants in this program is evident from the individual progress reports listing research publications which average more than four per individual during the past year. The progress reports also include summaries of academic and service functions being performed by the investigators which are complementary to the purpose for which these grants are made.

An analysis of the current research project grants and the Research Career Development Awards supported by the NIEHS shows roughly the following percentage breakdown: mutagenesis and reproductive toxicology 19 percent, etiology 33 percent, pharmacology-toxicology 40 percent, and pathology-pathogenesis 8 percent. The relative percentages for each major program area have remained fairly constant over the past several years and reflect to some degree the response of the academic community to the perceived needs for research in environmental health sciences. Institute-initiated programming in specific areas has not appreciably altered the relative size of the areas being supported, as indeed, it should not. However, over a protracted period of time in which programming efforts will be intensified as a result of the recommendations of the Second Task Force for Research Planning in the Environmental Health Sciences and the current program planning exercise, these relative percentages may be expected to vary according to programmatic directives. Although there is a need to continue to expand all program areas now supported which bear upon the environmental health sciences of concern to NIEHS, relevant Task Force recommendations demonstrate a need to strengthen certain disciplines by preferential

funding and programming efforts. The broad areas in which additional research are indicated includes: epidemiology, pathogenesis, behavioral toxicology, and improved methods for prediction of environmental disease. The latter involves all aspects of forecasting technology, including the means for identification of sources and understanding dynamics of transport and conversion of contaminants as well as exposure patterns. Some of the latter, however, may more appropriately be within the purview of other agencies.

The highlights of the regular research grants program follow much the same format of the previous year's report.

MUTAGENS AND CARCINOGENS

Studies included in the areas of mutagenesis and carcinogenesis include: identification of substances in the environment which may adversely affect the genetic make-up of man, development of reliable test systems, and research to elucidate molecular and cellular mechanisms in mutagenesis and reproductive anomalies.

Mutation Detection

Support is provided for a variety of research programs designed to explore the development of new assay systems to detect gene mutations and other types of genetic damage following exposure to environmental agents. The genetic damage of potential concern to humans include: chromosome rearrangements, gene mutations, abnormal chromosome numbers, inhibition of DNA repair and initiation of unscheduled DNA synthesis, mitotic recombination and gene conversions. The assay systems being explored include the use of micro-organisms and a variety of eukaryotic organisms, Drosophila, mammalian cells and cell cultures including Chinese hamster ovary cells, umbilical cord cells, and lymphoid cell cultures. Most of the systems using mammalian cells rely upon somatic cells since the use of germ line cells as test objects is much more difficult and less precise.

As mentioned above, Drosophila is one of the organisms which is being studied to determine its capability of identifying suspect chemicals that might pose a genetic threat to man, and to determine whether this species will be a reliable and realistic model for assessing the effects of environmental agents. To date, Drosophila is the only in vivo animal system which enables the efficient testing of both transmitted gene mutations and chromosome aberrations in germ cells. A number of tester stocks of Drosophila have been developed to provide a series of genetic tests from the same treated individual; thereby permitting a comparison of genetic response for one test as, for example, sex-linked recessive lethal versus translocations. Strains of Drosophila melanogaster have recently demonstrated that there is a high correlation between the mutagenicity of an agent and its carcinogenetic potential. It would appear from preliminary studies that the Drosophila species could perhaps serve as a model for monitoring the environment for the presence of mutagenetic substances. One approach to the use of Drosophila melanogaster to develop an assay system for mutagenesis takes advantage of the mutator genes and factors, such as, male recombination elements in the species which are often found in natural populations. There is still little information about

their frequencies or the effects they have upon the genetic structure of populations, however. A project is underway to investigate essentially three concurrent phases of the frequency and impact of mutator factors. A series of effects including mutator activities, segregation distortion, sterility, unique chromosome rearrangements and somatic mosaicism have been correlated with male recombination by a variety of workers. Preliminary studies have suggested chromosome breakage as a likely mechanism to explain these varied phenomena, and laboratory studies have been designed to test this hypothesis and to identify the causative agent. A further proposal is to develop and test a method of using quantitative variables to assess the effects of mutator genes and environmental mutagens.

In another study, Drosophila is also being used to study the nature of radiation-induced genetic damage. This is being carried out by testing the hypothesis of preferential elimination of radiation-induced damage by loss of one of the first cleavage products in Drosophila. The method involves the radiation of spermatozoa after a previous treatment with a chemical mutagen. If a chemical mutagen induces point changes which can appear as mosaic changes in the progeny, a subsequent treatment with radiation will induce lethal changes which will then convert the mosaic changes to whole body mutants.

A study designed to establish the procedures to be used in detecting chromosomal aberrations in a eukaryotic microorganism which would be suitable for large scale screening of drugs and chemicals is being carried out. A strain of Aspergillus is being used for the detection during mitosis and meiosis of recessive mutations, non-disjunction, crossing over, and translocations. It has been shown by these studies that ethanol causes chromosomal aberrations in the test organism. Findings are thought to be significant in light of the epidemiological correlations between esophageal carcinoma and ethanol consumption. Studies are continuing on other commonly-used drugs.

A number of host-mediated assay systems using cultured human lymphoid cells as target cells for screening chemical mutagens or carcinogens have been developed. Induction of damage in the chromosomes of the target cells are used as an indicator of possible mutagenicity or carcinogenicity of environmental agents. A variety of criteria is being used as an indicator of observed genetic effects with those measured by unscheduled DNA synthesis (UDS). The findings in the latter studies have shown that eight out of fifteen carcinogens induced UDS in the presence of a metabolic activation system and three yielded positive responses even in the absence of metabolic activation. The negative results included those from the tests of some polycyclic hydrocarbons, a class of compounds of concern relative to the potential hazard of increasing energy production and technology. In order to further develop the use of UDS as an efficient and reliable means of predicting the effects of hazardous agents on humans, an effort will be made to define one or more metabolic activation systems that will be more effective for use with UDS testing. It is hoped that after further refinement in vitro, unscheduled DNA synthesis most likely will be an efficient and economical system for predicting human susceptibility to hazardous agents including multiple agents acting synergistically. It may be possible to monitor human populations for adverse effects using UDS; however, the feasibility of this will depend upon additional experimentation. Another model test system for

monitoring environmental agents for mutagenic activity utilizes mammalian lymphoid cell cultures. Most eukaryotic cell work has employed as a positive control, the Ames S. typhimurium assay system. Recently, a short-term microbial system for mutagenesis testing has been described. This new system which is compatible with microsomal activation, involves the lysogenic induction of bacteriophage lambda in an E. coli strain deficient in cell wall and DNA repair. Various classes of chemical compounds have been tested in this assay system and the results compared simultaneously with the S. typhimurium and the polymerase deficient E. coli systems. The usefulness of the new assay system is currently being evaluated. In addition, a new host-mediated assay system for microbial mutagenesis is under investigation. The indicator organisms are not injected into the host and the active metabolites are removed by a modified form of peritoneal dialysis with a hypertonic dialysate solution. Using this system, activation of a number of carcinogens requiring activation, for example, benzopyrene and cytoxin, have been shown. In addition, the mutagenic activity of safrole in urethane has been demonstrated, and this was not possible with any of the previously described host-mediated bacterial assay systems. The same investigators have also shown that protein deprivation enhances the activity of those microsomal enzymes necessary for activation of 2-fluorinamine. Using selected media, a series of mutant human lymphoid cell lines have been isolated and work has begun on estimating the mutation rate at 3 loci: the HPT locus, specifying HGPRT activity; the AS locus, specifying argininosuccinate synthetase activity; and most recently, the O locus, specifying ouabain responsiveness.

In other studies, post-mitochondrial fractions from human lymphocyte and mouse myeloma cell lines have already been prepared and are being tested for their capacity to activate the pro-carcinogen to fluorinamine. The significance of these studies is that both the human lymphoid and the murine myeloma systems show promise of providing a test system for mutagenicity at specific loci in mammalian cells. In one such mammalian assay system, several common biological stains have been found to be strong clastogens. These include: quinacrine mustard, acridine orange, neutral red, and particularly gentian violet. It is felt that these agents should be handled in the same way as other biohazard chemicals, in view of these findings.

Another assay system involves the use of Chinese hamster ovary cells. This system differs from most mammalian cell mutagenesis studies in two aspects. One - instead of studying the genetic effects of agents at the single locus, many different gene-product variants are looked for within a single cell; secondly - the system can distinguish between genetic changes which modify the gene product and those which alter the expression of a locus. The cells from the Chinese hamster ovary are exposed to the mutagen, allowed to go through two divisions to segregate out mutant strains and then cloned randomly. Each clone is grown to 60×10^6 cells. A sample is kept in culture while the remainder is homogenized and supernatants are subjected to electrophoresis and the gels are histochemically stained for the products of over 50 enzyme loci. Structural gene mutations are viewed from the electrophoretic shifts in the product of an enzyme gene locus of a clone, and inherited in the sub-clones. Decreased, or, more significantly, increased activity of an electrophoretically separable isozyme of a clone, which is shown to be inherited in

sub-clones, is evidence for a mutation at a gene-controlled locus. The major emphasis in the past year has been quantitative characterization of the effectiveness of the system in recognizing mutagens. Various doses (1 percent to 50 percent survival) of ultraviolet light were used. Five hundred and forty-nine UV exposed clones were isolated and screened for a total of 27,177 enzyme loci products (the sum of all the loci over all the clones). These data indicate that the system is extremely sensitive in detecting mutagen-induced changes in mammalian cells. Techniques for pre-selecting cells for some drug-resistant marker and then looking for electrophoretic variants in them have been perfected. This allows not only the selection of double mutants but perhaps an increase in the system's resolution since only cells known to be affected in some way (drug resistance) are screened for electrophoretic shifts. Thus far, nine 6TG clones have been picked and analyzed for electrophoretic mutations. One has been found to have such a variant and has been successfully cloned.

Another study has been carried out to understand how environmental agents acting singly or in combination, damage the mammalian genome and how the cells repair such damage. Specifically studied was caffeine and/or its metabolites affecting the synthesis and utilization of purines and pyrimidines and how purine and pyrimidine synthesis relates to genome repair and survival of cells exposed to environmental agents such as ultraviolet irradiation. Preliminary experiments with caffeine strongly suggest inhibition de novo of purine synthesis at an early step in the pathway. The results are consistent with the working hypothesis that caffeine may act to enhance UV killing by limiting the availability and utilization of purines and pyrimidines. Caffeine, to which most of the human population is exposed, is thought to potentiate the damaging action of many physical and chemical agents found in the environment, including UV light and ionizing radiation. This damage may include embryo toxicity, teratogenesis, carcinogenesis and mutagenesis. The findings that caffeine interferes with purine synthesis may help to illuminate how caffeine increases cell destruction and chromosomal damage.

A series of experiments have been carried out to determine the dose response curve for a number of alkylating agents, in order to compare the genetic response to alkylation from ethylating and methylating agents that have high intermediate and low Swain-Scott substrate values. A new experimental design was devised for comparison of DNA alkylations among different alkylating agents. It was evident in the case with ethyl methanesulfonate (EMS), that there is a nonlinear relationship between concentration in the feeding media and alkylations of DNA sperm cells. Preliminary evidence suggested the value of about 8 percent sex-linked recessive lethals at the 2.0 millimolar feeding concentration for 24 hours, thus permitting a determination of a dose response curve over a range of response varying by a factor of about 40. This range permitted an accurate estimate of the dose-response relationship. The importance of distinguishing between exposure and dose is illustrated in that methyl methanesulfonate (MMS) is a more effective alkylating agent per unit of exposure than EMS, whereas EMS is more mutagenic per unit of dose (alkylations per nucleotide) than MMS. That MMS is the more effective alkylating agent is consistent with the faster rates of reaction of MMS with nucleophilic targets while the higher incidence of mutations per alkylation of EMS is

consistent with the lower S value of EMS and therefore, higher proportion of the alkyl groups on the O-6 of guanine and other ring oxygens.

Two specific approaches are being used to study DNA repair in man. The first is to isolate DNA repair deficient mutants in Chinese hamster ovary cells and use them to locate the corresponding human genes by somatic genetic techniques; and secondly, to continue to characterize biochemically as much as feasible, the molecular basis of DNA repair defects in several human genetic diseases. The class of human birth defects selected for study has a common feature of being radiation sensitive. They are manifested by chromosomal aberration and cancer predisposition. During the first part of this study, more than 40 potential mutants were isolated for future testing. Currently, additional procedures for assay are being developed which will be used to examine cross-links, pre-radical scavengers and metal toxicity. Since very little is known about repair on DNA lesions in humans, as well as in other eukaryotic cells, information generated from this study should contribute significantly to the understanding of these processes. With this type of understanding, one should be able to better define and avoid environmental factors that cause irrevocable DNA damages.

PHYSICAL FACTORS

Studies on the health effects of physical factors in the environment are becoming of increasing importance and interest. It is clear that additional research is needed on the health effects of microwave radiation, various radio frequencies, power densities which emanate from high voltage transmission lines, dose response relationship of ultraviolet radiation, and basic and applied studies on noise effects. There is an additional need to define and understand the relative susceptibility of various age groups to all of the above factors.

Noise

One research program attempts to specify qualitative and quantitative relationships between the physical characteristics of noise and the resulting noise-induced hearing loss and to evaluate the validity and range of application of the equivalent power model of noise-induced hearing loss. During the past year, an attempt was made to specify the relationship between the threshold shift and the level of the noise for octave bands spaced across nearly the complete range of audible frequencies. In addition, masking data and evoked responses were obtained in an attempt to localize the region in the auditory system where psychophysical-turning curves might be affected. An attempt was also made to predict the threshold shifts produced by wide band noise from the data described above. In the experiment, groups of human subjects were exposed for eight hours on consecutive days to a tailored, wide band noise. This noise was composed of octave bands centered at 0.5, 1.0, 2.0, and 4.0 kHz. A weighted sound level of the exposures which were held on separate occasions were 91.5, 87, and 81 dB. The 81 dB exposure produced threshold shifts of about 10 dB. For the 87 and 91.5 dB exposures, threshold shifts near 4.0 kHz were independent, or nearly so, of the level of the noise. The major difference between the 87.0 and 91.5 exposures was in the spread of threshold shift along the frequency domain; the higher the noise level, the greater the spread.

Using the chinchilla as an experimental subject, a study was made of the potentiating effects that various ototraumatic agents have on hearing. The ototoxic agents studied included impulse noise, continuous noise, sodium salicylate, and vibration. Hearing thresholds were monitored using evoked response audiometry (an EEG measure) and behavioral audiometry. Histological analysis relied primarily upon the surface preparation and sections of the sensory epithelium of the cochlea. Combinations of safe, spectrally overlapping continuous, and impulsive noise exposures have been shown to have a potentiating interaction. The present study was designed to determine whether matching of the impulse and continuous noise spectra is a necessary condition for the interaction effect. It was concluded that the spectrally mismatched combination has an interaction with increased traumatic power, but the effect is not as great as when the spectral distribution of the impulse and the background noise is more closely matched.

Microwave Irradiation

The measurement of modulated and pulse transmission of two frequencies of microwave irradiation are being used to determine the dosage and monitoring procedures through refinement of techniques and instrumentation. In addition, the determination of exposure levels that do, and do not, result in embryopathy, following chronic exposure of pregnant rats to low dose levels of microwave irradiation, is being carried out during pregnancy, during the implantation period, during the organogenic period, and during the fetal period. The specific objective of this research is to determine possible increases in rates of embryotoxicity or abnormal offspring, and, to determine how the threshold dose may vary with different types of microwave exposure and different embryopathies. Additional goals of the program are to determine whether the reproductive performance following in utero or adult exposure, is a sensitive measure of microwave exposure and to determine whether the central nervous system will manifest functional or architectural alterations following in utero microwave irradiation. The parameters chosen for study of irradiated and controlled mice include: maternal weight changes and mean litter sizes, resorption rates, abnormality rates, term fetal weights, and post-natal weight gains throughout life. No significant differences were observed between the groups studied. Reflex tests have been performed on offspring and controlled litter, including surface righting, auditory startle, eye opening, air righting, and visual placing testing. Again, there were no significant differences between the two groups tested. Behavioral tests included shuttle-box, water t-maze, swimming, open-field, hanging and activity wheel testing. These tests were completed for all the base-line controlled groups including a total of 479 tests. Initial comparison of the data indicates no significant differences among all groups tested utilizing these six tests. The significance of these tests is in the development of a sensitive means of determining the degree of irradiation in consideration of the exposure of the general population to governmental, industrial, and commercial sources of chronic low levels of microwave irradiation.

Radionuclides

Studies are continuing to evaluate the health effects on local or global populations resulting from dispersion of nuclides from the nuclear power

industry. The nuclides which are under most vigorous attack are the long-lived alpha emitting isotopes of plutonium, americium and curium. In addition, the gaseous beta emitting fission product K-85 is entirely released during fuel reprocessing and is globally dispersed. For all nuclides, the most important dose estimates are to cells in bronchial epithelium and cells on bone surfaces. The long-lived alpha emitters will almost certainly be dispersed during the fuel reprocessing, however, their effect is local and not global and at worst, a single community would be affected. The most important pathway for exposure to the actinides is through inhalation following resuspension of contaminated soil particles. Additional exposure is through absorption after material is cleared from the lung and swallowed. However, absorption from the GI tract is small and the primary dose is to the lung. The cell doses will be estimated for a reference contamination level of soil for each nuclide.

Thermal Adaptation

Studies have been carried out on the thermoregulatory capacities of females performing light, moderate, and heavy work under conditions of heat stress. As a result of these studies which concentrate on the interaction of age, cardiovascular fitness and the menstrual cycle with exercise in the heat, it has become apparent that most of the prior literature comparing male and female response to work under heat stress suffered from faulty experimental design. The results of these studies indicate that the ability of females to tolerate work in hot environments was underestimated in the past, primarily because male/female comparisons were not made at the same relative work load, nor was cardiovascular fitness considered in the experimental design. Women respond well to intermittent work in the heat, when the rest period is in a cool room, responding primarily to the metabolic requirements of the task rather than to the environment. Their responses were directly related to a weighted mean of metabolic requirements and ambient conditions. While the final analysis of the menstrual cycle effect is not yet complete, the initial data, plus two published studies indicate that the cycle plays a minor role in women's ability to tolerate heat. A more important variable is age. The data suggest that both pre-pubertal and post-menopausal females are at greater risk than other segments of the female population. The basic mechanisms responsible for these differences remain unexplained, however.

Odorant Irritants

The objective of the study is to characterize how human beings perceive the intensity of odorants and irritants under various conditions of stimulation. The project focuses attention on: (1) the relative contributions of the olfactory and trigeminal nerves to odor magnitude and irritation, (2) the efficacy of odor counteractants and masking agents, (3) the resolution of small differences to intensity, and (4) adaptation. Experiments are designed to characterize the functioning and to uncover the mechanisms of the olfactory and common chemical senses. Success of this endeavor may have broad implications for the understanding of sensory processes in general. Potential applications of the research lie in the areas of industrial, and environmental health and safety. Sensations aroused by chemical vapors often signal the presence of hazardous situations and often cause discomfort. Characterization of these sensations

may offer some opportunity to minimize the discomfort and to maximize the effectiveness of warning agents.

Metals

Cadmium is used in electroplating, in the production of alloys and pesticides, and in pigment formulation. Motor vehicle exhausts and industrial effluence contribute to the atmospheric burden of cadmium. Cadmium is a by-product of zinc and lead production, and, due to the increased use of this metal, the potential for environmental exposure continues to grow, particularly in the heavily-industrialized areas of the world. A study is underway to elucidate the neurotoxic effects of chronic low level cadmium exposure including the role of essential trace metals. The relative effects of cadmium exposure on selected neurophysiological and behavioral parameters during the pregestational, neonatal and adult periods are being observed. Also being studied are the effects of various concentrations of zinc, copper and iron in rat dams and their pups. Exposure to cadmium during the pregestational period and gestational period have demonstrated that the presence of cadmium in the drinking water during the gestational period only, is as effective in promoting trace metal and behavioral changes in pups as exposures which include pregestational exposure as well as exposures during the gestation period. Exposure during the pregestational period only, had no detectable effects. From this, it appears the period of gestation is the critical time to cause increases in locomotor activity of pups. Aggression also seems to increase, and sensory and motor nerve conduction velocities in the sciatic nerve of the pups fail to demonstrate any deleterious effects in those animals previously exposed to cadmium. The latency and amplitude of responses recorded from the cerebral cortex evoked by sciatic nerve stimulation were also not altered by cadmium exposures. However, another index of central nervous system function, the recovery function of the inner hemispheric response, was depressed in cadmium-exposed rats.

The toxicological significance of cadmium-metallothionein (Cd-Mt) is being determined by experiments to learn how the compound is handled by different tissues. In the kidney, it was found that renal filtration is followed by reabsorption of Cd-Mt and therefore, this mechanism is insignificant in cadmium excretion. Tubular secretion of Cd-Mt was also found to be insignificant or extremely slow; therefore, it appears at this point that complexation of cadmium with metallothionein is not a mechanism by which the organism enhances the urinary excretion of the toxic metal.

Another study concerning metallothionein involves the establishment of an in vitro cadmium binding protein (Cd-Bp) synthesizing system. The material can be purified by chromatography and is identical to standard Cd-Bp synthesized in vivo. It has been demonstrated that although the cadmium-protein complex is degraded over time, the released cadmium is rebound to more newly synthesized metallothionein chains. This may explain why, in spite of this degradation, cadmium is retained in target tissues for extended periods. Cadmium retention was also found to be enhanced by feeding low-calcium diets because the low calcium stimulus activated release of parathyroid hormones which, in turn, enhanced calcium and cadmium absorption.

The mechanism of action of the cadmium induced limb malformations in mice is being pursued. The maximum sensitivity was found to be on day nine of pregnancy and individual treatment of both parents has indicated that the effect is entirely maternal. The teratogenic action of cadmium in susceptible mice shortly before pregnancy, markedly reduces the teratogenic effect of cadmium administered on day nine of pregnancy. It is, therefore, hypothesized that cadmium pretreatment causes the induction of some protective protein. The teratogenic effect of inorganic lead in the hamster is also being demonstrated. Intravenously administered lead nitrate on the eighth day of the 16-day gestational period, showed microscopically visible signs of developmental disturbances in the examined embryos. A pronounced and highly site specific malformation of the sacral-tail region was observed and it was felt that this related to the peculiar pattern of embryogenesis of the area.

The chemistry, biochemistry and physiological chemistry of the metallothionein is being investigated to clarify its role in essential metal metabolism and heavy metal toxicity. Using Erlich tumor cells injected with cadmium chloride CdCl_2 a 10,000 molecular weight cadmium binding protein has been identified. Interestingly, about 50 percent of the cellular Cd is found in the particulate fraction of the cell, paralleling the findings of the rat liver. These studies have also shown differences in the turnover of these metals in organs. The difference is that while both the zinc and cadmium are taken up into cells, in fresh metal free media, zinc has an efficient efflux mechanism, whereby cadmium remains bound in the cell. It is felt that the studies with livers from rats on diets containing cadmium and those studies involving tumor cells exposed to cadmium will contribute to our understanding of how cadmium interacts with cells and what role metallothionein plays in these reactions.

In another study, time courses of induction of metallothionein and chelatin in rat liver under in vivo conditions were carried out for cadmium, zinc, and copper induction. The goal of these studies is to obtain a better understanding of the processes involved in heavy metal metabolism, particularly focusing on the unusual protein metallothionein and its involvement in the in vivo metabolism of metabolically essential and toxic heavy metals. In a later work, a single intraperitoneal injection of mercuric chloride led to the synthesis of zinc thionein in rat liver with a peak of induction at 18 hours and a $T_{1/2}$ of 9.4 hours, similar to that which was seen with zinc induction. No mercury was found to be attached to the purified metallothionein after induction by mercury. Actinomycin D administered at the peak of induction led to a two-fold increase in the level of zinc thionein, 12 and 23 hours afterward relative to controls receiving no inhibitor, which was reminiscent of "super induction". The behavior of the induction of zinc thionein in rat liver by mercury is similar to that seen previously with induction by zinc and copper but is different from that seen with induction by cadmium. The production of a rabbit antibody monospecific for rat zinc has successfully been elicited. Full development of a radioimmunoassay for rat liver thionein is currently nearing completion.

The ubiquitousness of lead in the environment and its associated health problems continues to be of interest to environmental health scientists. The major uses for lead to which human populations are exposed include storage

batteries, fuel additives and pigment manufacture. Chronic or subacute lead exposure leads to neurological, renal and hematological manifestations of disease.

In one such study, in anticipation of the expected increase in the lead levels in the environment, the effects of lead intake by children and the adverse effects of lead accumulation on neurophysiological development are being pursued. Utilized in this study are longitudinal neuropsychological, behavioral, and biomedical data collected prospectively, and assessment of current neuropsychological functioning. Analysis of lead content in the primary and secondary dentine of the subject's exfoliated deciduous teeth are providing an index of lead accumulation over time. By integrating these two sets of data, attempts are being made to determine whether lead exposure at various ages, singly or in concert with other developmental events, produces deficits in neuropsychological functioning in children. These studies utilize atomic absorption spectrophotometry for lead analysis of the teeth.

Since the bone is the reservoir for the majority of the body burden of lead, factors which control the metabolism of lead are particularly important to understand, since lead may be mobilized from bone during chelation therapy and during rapid skeletal remodeling characteristics of growing children. A project to define various factors which determine the movement of lead into and out of bone, such as hormones, ions and vitamins is being carried out. Metal-hormone-vitamin-nucleotide-collagen interactions are being studied in bone organ culture and studies in parathyroidectomized rats are being used to test the relevance of the cell culture findings.

In culture, it was determined that in addition to its chelation of lead, ethylenediamine tetraacetic acid (EDTA) produces solubilization of bone material and matrix qualitatively similar to the actions of parathyroid hormone. After brief exposures to parathyroid hormone or EDTA, and subsequent transfer to medium without either agent, the release of lead continued, and was virtually the same as that released in continuous culture with either agent for protracted lengths of time. Hence, EDTA can induce lead release after brief exposure. Lysosomal mechanisms were also determined to play an important role in the release of previously incorporated lead from bone into the medium. Agents such as glucocorticoids and Vitamin A had marked defects upon lead transport. In parathyroidectomized rats infused with EDTA or parathyroid hormone, it was determined that parathyroid hormone produced an increase in brain-lead content. On the other hand, calcitonin inhibited EDTA's action by blocking removal of lead from bone. Clinically, preliminary evidence was developed that children with mild to moderate absorption of lead, have low circulating levels of dihydroxy Vitamin D compared to normal controls. Thus, the enhancement of lead toxicity in children with marginal nutrition is suggested.

The pathogenesis of the encephalopathy resulting from exposure of the immature brain to inorganic lead is under investigation. Specifically how lead effects mitochondrial energy metabolism is being studied in mature and young rats. It has been observed that the effects of lead on cerebral mitochondrial respiration were confined to the first two weeks of life and appear to be independent of decreasing packed cell volumes and increasing brain-lead concentrations which change during four weeks of feeding of lead carbonates.

The effects of lead acetate on isolated rat brain mitochondrial respiration were determined to be an increase in respiration coupled to oxidative phosphorylation at low lead concentrations; whereas higher lead concentrations inhibited both lead-induced and ADP-dependent respirations. NAD-linked citric acid cycle dehydrogenases, located in the mitochondrial matrix, were more sensitive to lead inhibition than were the inner membrane enzymes. These in vitro effects were similar to those seen in cerebellar mitochondria from rat pups fed lead from two weeks of age, and were the same in cerebral and cerebellar mitochondria from two-week old and adult rats. Since the rats serve as a useful model to study the biochemical pathogenesis of the effects of lead on the immature human brain, the predominantly cerebral effects of lead in the newborn rat suggest that it could be important in the pathogenesis of cerebral dysgenesis or unexplained mental retardation in humans.

Studies on the effects on lead of calcium movements across the blood brain carrier are also being supported. It has been determined that lead intoxication disrupts the transport of calcium at the blood brain barrier. This demonstrates that lead and calcium may interact at various points in the membrane system constituting the neural barriers. These observations may have significance for understanding the pathogenesis of lead encephalopathy since so many processes of importance in neurofunction, such as neurotransmittal release, are calcium-dependent or associated.

Studies on the potential teratogenic effects of heavy metals are being supported. It has been found that arsenic ion produced specific malformations in surviving offspring of hamsters, if injected during certain critical periods of gestation. It has been determined that arsenic crosses the placental barrier and therefore, may be a direct cause of the teratogenic effect. Furthermore, hyperthermia, although itself a condition which can cause malformations, is synergistic with subteratogenic dose levels of arsenic.

Investigations are continuing on the study of mercury in the environment and the mechanisms of mercury toxicity in animals and man. One such study attempts to determine the minimum level at which organic mercury can be toxic for the primate fetus and the neonate. Thus, the extent of transplacental passage of various forms of mercury and mercury vapor in the monkey are being determined and also the levels of mercury in the fetal and neonatal tissue following maternal or direct exposure to mercury. The localization of mercury within the brain is being determined and the nature of the resulting damage to various brain regions. Finally, the blood levels of organic and inorganic mercury in a population of human mothers and their children are being correlated with maternal diet, age, parity and the environment.

Treatment regimes for methylmercury intoxication are being evaluated by comparing their effectiveness against the toxic effects of methylmercury, chloride, bromide, iodide, hydroxide, or other salt, and against the effectiveness of agents presently used for such treatment. The initial step, the definition of the toxicities of these various salts of methylmercury, is under way. Lethal doses and behavioral teratologies are being defined and a comparison of various treatments on these effects indicates differences in efficiencies of treatments to attenuate the toxic effects. In addition,

pharmacokinetic parameters (absorption and distribution) of methylmercury have been determined.

A knowledge of the amount of Polonium-210 in the lungs of cigarette smokers is needed in order that this alpha emitter is to be implicated in smoking-related carcinogenesis. It has been suggested that inhaled PO-210 present in main stream tobacco smoke, can be a contributor to the formation of bronchial tube tumors. The amount and distribution of isotopes of lead and polonium in smoker's lungs over non-smoker's lungs is being determined. Additionally, the solubility of polonium in lung fluids is being determined. The extent to which the inhaled polonium is directly retained in the lung is difficult to estimate since some polonium is cleared to the blood and systemically re-distributed. To resolve this question, lung samples are separated into tracheobronchial tree and parenchyma for radiochemical analysis. Preliminary results indicate that there is a significant difference in the ratio of the concentration in the tracheobronchial tree to that in the lung parenchyma.

Study is being made of the mechanism of carcinogenesis by nickel compounds. Specific objectives of the study include the elucidation of the mechanism of manganese inhibition of Ni_3S_2 carcinogenesis, identification of specific cellular and molecular alterations that occur during nickel carcinogenesis utilizing differences in the carcinogenicity of Ni_3S_2 and Ni S , and to characterize an experimental system for induction of renal cancers in rats by intrarenal injection of Ni_3S_2 . The results of the studies, to date, have shown that manganese antagonism of Ni_3S_2 carcinogenesis in rats is a local phenomenon rather than a systemic effect, that manganese antagonizes carcinogenesis by benzo(a)pyrene, as well as Ni_3S_2 and that manganese suppresses the erythrocytosis that is induced in rats by intrarenal injection of Ni_3S_2 and finally that manganese diminishes the incidence of renal cancers induced by Ni_3S_2 . Moreover, it has been shown that Ni_3S_2 induces morphological transformation of primary cultures of hamster embryo cells in vitro, that this transformation may be prevented by manganese and that ^{63}Ni becomes bound to three distinct proteins in the ultracentrifugal supernatant fraction of renal homogenates of rats after ip administration of $^{63}\text{NiCl}_2$. The most significant findings are the anti-cancer effects of manganese dust and the apparent accomplishment of in vitro transformation of tissue culture cells exposed to Ni_3S_2 .

A variety of other studies are underway to investigate the role of other metallic substances in relation to enzyme systems which are of significance to the basic understanding of human health. These include selenium and its role in the hepatic microsomal cytochrome P-450 system and the identification of selenium containing proteins and liver microsomes. The pathway whereby human cells assimilate selenium into the enzyme glutathione peroxidase is being studied. Lymphocytes are employed as the test system for the uptake of selenite. The specific enzyme activity of glutathione peroxidase from human lymphocytes is five to seven times greater than that found in whole blood hemolyates. These cells, therefore, offer a good experimental system for studying the incorporation of radioactive selenium into the system.

AIR POLLUTANTS

A wide variety of human activities generate respirable particles, many of which remain airborne for extended periods of time. However, the health implications of these particles are often unknown, and evaluations must be based upon estimates of uptake from the inhaled air, subsequent deposition, and fate of the retained material. Current predictive deposition models are, at best, simplified approximations; when their predictions are compared to actual deposition measurements, these models have been found to be inaccurate and inadequate. Development of better models requires clarification of the quantitative aspects of regional particle deposition patterns within the tracheobronchial tree. Of importance then, is an understanding of the amount and pattern of particle deposition within the human tracheobronchial tree, for respirable particles of various sizes, under a variety of normal breathing patterns, and the development of reliable predictive deposition models for inhalation-hazard evaluations. The role of alveolar macrophages and other similar cell types in the lung in the defense of the body against noxious airborne particles is still not fully understood. However, since these cells form the cornerstone of alveolar defense, any contribution to the understanding of their operation is relevant to an understanding of the health and disease state of the lungs. Therefore, studies are focusing upon the varied aspects of macrophage behavior that are critical to the defense of the body and to a healthy state. Some of these factors are phagocytosis, the response of the macrophage digestive system to bacteria and particles, factors influencing macrophage recruitment, maturation, clearance of particles, etc.

A study on the distribution and retention of inhaled pollutants to determine aerosol deposition in the respiratory track has as its goal the quantitative assessment of regional deposition in the human respiratory track. Using dogs as experimental models, aerosols of known particle size distribution have been prepared with enough radioactivity for scintillation imaging, upon inhalation by the experimental animals. The procedures for particle development, measurement and inhalation have been successfully developed, and carrier gas characteristics have been defined. From initial studies, it has been hypothesized that aerosol deposition and turbulent flow is created by obstructive changes in the airways. Radiographic aerosol spots appear to be directly related to localized turbulent flow. This would cause increased deposition in patients with a variety of chronic obstructive lung diseases and indicate an increased susceptibility of these patients to the effects of environmental aerosols.

A two-component theory of aerosol transport and deposition in the lung has been developed. This two-component theory assumes that the air mixing occurs largely within the alveolar airways. This is contrary to the previous belief that mixing occurs only in the upper airways. To support the assumption of the two-component theory, a theoretical study was undertaken on a model alveolus based upon the basic fluid mechanical principle. It was found that the air mixing can indeed occur within the deep lung due to the fact that the alveolar shape changes during breathing and that the inhalation and exhalation are not mere image processes. Quantitative calculations based on both steady breathing and single breath washout were made. For steady breathing, the total and

pulmonary deposition results for particle size between 0.1 millimicron and 10 millimicron aerodynamic diameter compared very well with existing experimental data compiled by other investigators.

The factors which influence deposition of inhaled particles are being investigated using new techniques involving the use of a sophisticated gamma camera, autoradiography, and the analysis of lung pieces. The experiments employ a servo-pressure control, and shapes of breathing patterns can be selected. These studies are continuing to measure and map the pattern of inhaled particle deposition.

One particulate air pollutant of extreme interest at the present time is asbestos and its relation to cancer. Although exposure to large amounts of asbestos has been definitely associated with the development of lung cancer, the magnitude of the risks has not been defined in persons who are exposed to relatively minute doses of asbestos. Small amounts of asbestos together with cigarette smoke, have been shown to have a synergistic effect in producing pulmonary carcinoma. In a preliminary study, using techniques to extract and quantify asbestos bodies in lungs, high numbers of bodies were found more frequently in lung cancer patients than in a controlled-autopsy population. This finding will be confirmed by comparing the number of asbestos bodies in the lungs of a large series of lung cancer patients with a more closely matched controlled group. Similar studies will be performed for other cancers in which asbestos is etiologically implicated: carcinoma of the esophagus, stomach and colon. Seventy-three lung cancers have been collected and the donors matched for age, sex and smoking habits. Preliminary results indicate that there is no association of lung cancer with asbestos bodies at these low doses, although there is an association of cancer with occupation. The number of bodies does not appear to correlate with occupation and smoking habits. Lungs from 35 patients with gastrointestinal cancer have been collected and matched in a similar way. Again, no correlation of asbestos bodies and cancer was apparent. Asbestos bodies were quantified in digests of lungs from 252 urban patients over 40 years of age. Patients were assigned to six occupational categories. Asbestos bodies were present in the lungs of 95 percent of the total population. Fewer than 10 percent of the white-collar males, and blue and white collar females had more than 100 bodies per gram of lung; whereas 33 percent of steel workers and 61 percent of construction workers, had more than 100 bodies per gram. This distribution suggests that there is a background exposure to asbestos in the environment for everyone in the population.

A model system for the study of regional deposition of inhaled particles in man is under study using an artificial larynx which has been constructed and which simulates normal inspiratory flow testing, mimicking in vivo the laryngeal movements. The overall objectives of the project are to determine the quantitative characteristics of regional particle deposition within the conductive airways of the human lung, the variation which occurs among individuals and with locations along the airways, and the effects of variations in particle characteristics in respiratory parameters on deposition patterns. An earlier finding has shown that the larynx plays a significant role in aerosol deposition and that the toxic doses resulting from the deposition of inhaled particles depends upon many factors. Among the most important, but the least

understood of these, is the pattern and efficiency of deposition in the major conductive airways. The results of these studies provide, for the first time, a quantitative description of deposition in the larynx and bronchial airways in vivo and in realistic model systems, and provide better predictive relations for such deposition than were previously available.

Ozone is a photochemical oxidant and an important air pollutant which, by itself and in combination with other chemical substances in the atmosphere, has important implications for health effects, both in the acute and chronic exposures. It is known to damage cell membranes and denature structural protein. A number of research projects are being supported to better understand ozone toxicology. One such study has as its objectives: (1) the characterization by light microscopy, electron microscopy and morphometry, the growth and development of residual lung tissue in rabbits following unilateral pneumonectomy; (2) to expose pneumonectomized animals for variable periods of time to the common air pollutant, ozone; and to determine if, and to what extent, growth of residual lung is impaired by exposure to the gas and the factors that are responsible for this impairment; and (3) to compare the right lungs of animals following pneumonectomy with the left lung removed at the time of surgery following pneumonectomy, with the left lung removed at the time of surgery with the lungs from controlled animals. It has been shown that the effects resulting from a combination of pneumonectomy and ozone are a thickening of the air-blood barrier, a decrease in mean linear intercept (air spaced diameter) and a small amount of interstitial edema. These are changes which are not seen in pneumonectomy alone. Since the small airways play an important role in chronic obstructive lung disease, the knowledge of their structure and function in different animal species is important to an overall understanding of this disease process.

Another approach to the study of the effects of ozone on cellular processes involves the development of a system with which to study the toxicity of gaseous or volatile environmental toxicants on cultured cells of the lung. Specifically, rat lung macrophages and fibroblasts are cultured and exposed to the effects of ozone. The use of cultured lung cells would permit evaluation of specific cell types and the use of techniques that are not possible to evaluate under in vivo conditions. Such a system of cell cultures should provide further understanding of the toxic mechanisms involved and a means to minimize the toxic effects.

The hypothesis that the toxicities of ozone and nitrogen dioxide are related to their ability to initiate peroxidation of unsaturated fatty acids is being tested. By varying the dietary intakes of polyunsaturated fatty acids and Vitamin E, observing pulmonary levels of these materials and measuring the rates of peroxidation in the lung, an index of peroxidizability can be determined. In rats and in mice, morbidity due to ozone exposure is decreased by feeding large amounts of Vitamin E. However, no difference in mortality from exposure to 1.0 ppm of ozone was observed in mice fed 0 or 10.5 mg/kg Vitamin E. Further experiments are being conducted to determine the effects of Vitamin E on normal lung biochemistry and to determine if more complex performance tasks might be modified.

The effects of gaseous and particulate pollutants, such as, sulfates and sulphur dioxide on the systems which maintain lung sterility including the mucocilliary transports systems and alveolar macrophage-functions, are being determined. Rats and mice were exposed to defined atmospheres of sulfate particles and sulphur dioxide. Preliminary data indicate that the antibacterial defense mechanisms of these animals are, for the most part, unimpaired by exposure to combinations of these agents. The levels used in the experiments indicate that present air quality standards for sulfate and sulphur dioxide exposures are below that necessary to cause abnormalities. These experiments will be continued in order to determine the effects of chronic exposure to sulfates, sulphur dioxide, nitrates, and nitrogen dioxide.

An investigation is underway to determine whether electronically-excited-singlet-molecular oxygen exerts its damaging effects on tissues by oxidation of specific unsaturated lipid components of cellular membranes. At the present time, interest is being directed toward cholesterol and related unsaturated sterols as possible target molecules. Studies to date establish that several sterols can be used for detection of singlet oxygen in various systems and that chromatographic methods can be used for the analysis of the product mixtures which may result from such oxidations. Using these methods, it should be possible to determine whether a singlet oxygen does indeed oxidize sensitive lipid-rich membranes of exposed tissues.

An attempt is being made to correlate the natural history of Chronic Obstructive Respiratory Disease (CORD) and functional respiratory impairment with long-term exposure to different types and levels of air pollutants in residents of four demographically similar communities. In addition, the sensitivity and specificity of selected tests of lung function in identifying early respiratory impairment and chronic obstructive respiratory diseases are being measured. A determination has been made of the prevalence of chronic obstructive respiratory disease and functional respiratory impairment in one study-area and a comparison made of the prevalence of these disorders in populations exposed to minimal levels of ambient air pollutants, moderate levels of photochemical/oxidant pollutants, relatively high levels of hydrocarbon, SO₂ and low photochemical/oxidant pollutants. These comparisons will provide information on whether long-term exposure to different types and levels of ambient air pollutants plays a role in the natural history of chronic obstructive respiratory disease and functional respiratory impairment in these cohorts. Comparison of the results of earlier lung function testing in other areas suggests an association between lung function and pollutant exposures.

PESTICIDES/HERBICIDES

The key to the safe use of any pesticide is a knowledge of the basic toxicological data, based upon logical explanation of its mode of action and side-effects. The biochemical mechanisms of poisoning have not been fully understood for such environmentally-important agents as the chlorinated pesticides and newly-developed pyrethroids. They are, by and large, extremely lipophilic, but, chemically-unreactive compounds. Their major effects are upon the nervous system and the liver. One such study involves the effects of various insecticidal chemicals on ATPases. ATPases play

vital roles in just about every organ of the body and are responsible for a large portion of the total energy expenditure of an animal. Thus, studies such as these will aid in assessing the toxicological hazards, the development of antidotes, the synergistic action with other chemical compounds, and other key problems inherent in their use. The spontaneous reactivation of acetylcholinesterase (AChE) is being studied in an attempt to devise therapies for organophosphate insecticide exposures. Reactivation of AChE is temperature-dependent at the temperature at which inhibition by organophosphate is carried out. This result supports the hypothesis that AChE is rather long-lived, and therefore, lower temperatures would further retard its aging. Detailed temperature dependence studies have indicated that reactivation of AChE requires hydrolysis of an enzyme-inhibitor bond and a major enzymatic conformational change. The susceptibility of weanlings and newborns to organophosphate insecticides has been found to be due, in part, to incomplete development of the liver microsomal enzymatic systems which biotransform organophosphates. It has, therefore, been hypothesized that cases of fetal death and malformations might be related to poisoning by insecticides. Treatment of pregnant mice with parathion produced a significant incidence in deaths in utero and produced a significant reduction of body weight in surviving fetuses. The effect was dose-related. The highest concentration of acetylcholine found does not exhibit a significant affect on the placental vascular system. The role of acetylcholine is probably to regulate the transport of charged molecules across the trophoblast. This may explain how interference with the placental cholinergic system can play a significant role in the regulation and maintenance of fetal growth and development. Perturbations by organophosphates on this ion-transport system are under investigation to determine the causes of these responses.

The molecular basis of the action of selective insecticides, such as, anticholinesterase, chlorinated hydrocarbons, and pyrethroids is also under study. The effects of DDT and pyrethroids on membrane permeabilities to ions by affecting the "sodium gate" is being carried out using radio-isotopes. In addition to the specific information being obtained about the mechanisms of action of these insecticides, the molecular probes being developed to study the "sodium gate" may lead to a further understanding of the apparatus and shed light on the mechanism of actions of many neurotoxins.

The fates of photoisomers of cyclodiene insecticides in food chain organisms is being studied to relate these fates to ecological and health effects of the parent compounds and their residues. It has been determined that photo-generated isomers of dieldrin, cis-chlordane and heptachlor are several times more toxic than the parent cyclodiene to fish and to crustaceans. However, the bioconcentration of these materials does not appear to be a problem since they have been determined to be excretable by mammals and fish and therefore, are not likely to remain accumulated in the body after a single exposure.

Neurotoxic pesticides (organophosphorus esters) are being studied to increase our understanding of their mechanism of action and to evaluate the hazards of these agents to man and animals. The overall chemical structures of a series of substituted alkyl and aryl phosphonates, phosphonothioates, phosphoramidates, and phosphoramidothioates is under investigation. Oral dosing of hens is used in this evaluation. It has been determined that chlorine

or bromine substitution on aryl groups does not modify neurotoxic effects to any great extent. However, isopropyl configurations on alkyl chains are highly conducive to neurotoxicity. In addition, several of these compounds have been evaluated in model ecosystems for degenerative pathways in a number of organisms. In general, these compounds are environmentally persistent, stable and have the potential for bioaccumulation.

The mechanism of action of pyrethroid insecticides is under investigation to learn more about the chemical's action and the structure-activity relationships. Nerve impulse versus chemical structure studies have indicated a similarity between the actions of DDT analogs and the pyrethroids. Both appear to be more toxic at lower temperatures and both are thought to be active on the nerve membrane. The observations are being pursued in an attempt to acquire additional information on the modes of action of this class of compounds and for insect control.

The relationship between inhibition of tissue esterases and amidases by exposure to organophosphate insecticides and alterations in the metabolism, and toxicity of carboxylester- and carboxylamide-type drugs is being studied to determine the maximal level of esterase and amidase inhibition which can be tolerated without alteration in the action of toxicity of ester and amide drugs. Initial results with two organophosphates (EPN and Dasanit) in mice strongly suggest the potential for dangerous interactions with the local anesthetic drug, procaine. Toxic interactions occurred under conditions in which there was a minimal inhibition of brain cholinesterase. Apparently, nontoxic doses of organophosphate insecticides decrease the rate of metabolism and concomitantly increased the toxicity and lethality of this therapeutic agent. The potential importance of this observation with regard to medical treatment of individuals exposed to organophosphate insecticides is obvious.

An attempt is being made to clarify the mechanism by which 2,4-D influences transport of ions in lipid membranes. It would appear that 2,4-D enhances transport of cations and suppresses transport of anions. From the kinetic studies, it is concluded that the transport modifications are due to the change of a partition coefficient of membrane permeable species, between the aqueous phase and the membrane surface. All the evidence indicates that in the presence of 2,4-D, the membrane boundary potential difference changes and the membrane core region becomes electrically negative. Pentachlorophenol appears to function as an effective complex probe of sterol influences, since proton transfer across the interface is sensitive to conditions within the surface region, and admittance measurements provide information on surface transport. It would appear that strong sterol-induced modification in transport properties requires a beta-hydroxysterol with a flat nucleus and intact side chain; the presence of sterols can cause structural modifications in the bilayer surface. The relative effectiveness in modifying transport properties of sterols with slightly differing structures may depend upon the depth of insertion of the sterol into the membrane structure.

In another study, the objective is to establish the basic biochemical characteristics of major components of the insect microsomal enzyme system. It is hoped that through comparative studies with similar components of mammalian microsomes, differences might be uncovered which can be exploited

in the more rational design and development of new insecticides showing greater selectivity. Specific aims include attempts to solubilize, purify and characterize the major catalytic components (NADPH cytochrome C reductase and cytochrome P-450) of controlled and induced army worm (Spodoptera eridania) midgut microsomes, to study the role of DNA-independent RNA polymerases in microsomal enzyme induction in the army worm and to solubilize and characterize the microsomal epoxide hydrase in the same species. It is hoped that in-depth comparative studies of the type being carried out in this study will yield useful information in the design of safer and more selective insecticides.

The biological control of insect pests is becoming more of a reality with scientific advances providing impetus to programs of integrated pest management. The public health advantages of such programs, of course, relate not only to insect control, but also to a lesser reliance upon chemical pesticides with their potential for adverse health effects. One such program involves the synthesis of naturally-occurring anti-feedants including ajugarin-I, ajugarin-II, and warburganal, and the preparation of structural modifications of these materials for testing as anti-feedants.

The potential for the control of predatory insects through the use of natural agents is great and the studies directed toward the preparation of several natural anti-feedants and potentially-active modified compounds are close to the point of realizing their potential for insect inhibition.

Baculoviruses have been proposed as biological insecticides. A thorough knowledge of the process of baculovirus infection, the genetic information contained in the baculovirus DNA genomes, and the expression of this information, is necessary to establish the safety of using these organisms as insecticides. In the present investigation, a rapid and precise means of identifying baculovirus pesticides has been provided and demonstrates the need for cloning viruses before they are registered as pesticides. Restriction endonuclease (REN) "fingerprints" of a wide variety of baculoviruses have been obtained. The REN patterns are indispensable as a means of rapidly and precisely identifying the viruses. In addition, fingerprints of A. californica MNPV, a virus which is currently under consideration for EPA pesticide registration, have been worked out. Thus far, the study has provided basic information on the genetics of the organisms which will be of future importance in risk assessment and safety analysis.

In another study, designed to elucidate the biochemistry of the build-up and breakdown of chitinous insect cuticle, the ultimate goal is a rational design for development of specific insecticides and antimycotics and a substantial lessening of the environmental burden through their use. Evidence has been obtained from this investigation that chitin prepared from different sources have widely differing capacities to serve as substrate for the same or for different chitinases. The elucidation of chemical differences which must underlie these biological differences has begun.

ORGANICS

The chemistry and biological actions of dioxin (TCDD) and related compounds

are under investigation to determine the mechanism of action of these compounds at the molecular level. Whereas TCDD analogs are known potent inducants of aryl hydrocarbon hydroxylase (AHH) activity, the induction of AHH was initially used as an indicator of the compound's potency. Utilizing TCDD analogs and halobiphenyls, it was found that those halobiphenyls which induced AHH activity are recorded in the literature to be the most toxic, and the toxicities are qualitatively similar to TCDD. An additional similarity of strong binding to hepatic cytosolic constituents give further generalization as to the structure-activity relationships observed (all compounds are planar and roughly rectangular, with halogens in, at least, three of the four corners of the rectangle). These similarities indicate that the mechanism of toxicity may be mediated through a cytosolic binding protein.

Piperonyl butoxide, a widely-used pesticidal synergist, is being studied to determine its affect on mouse liver microsome-enzyme induction and on the metabolism of several carcinogenic chemicals. These effects will then be correlated with biological responses to hepato carcinogens, for example, DNA binding, liver cell necrosis and carcinogenesis. It has been observed that the mutagenicity of aflatoxin B₁ was stimulated by inclusion of microsomes from piperonyl butoxide treated mice and hamsters in the Ames test. Safrole also stimulated aflatoxin B₁ mutagenicity. Aflatoxin B₁ treatments also increased the number of dominant mutations and a synergistic effect was observed with piperonyl butoxide present in the diet. Piperonyl butoxide also stimulated AAF N-hydroxylase and increased AAF binding to RNA, DNA and protein in vivo. When microsomes from piperonyl butoxide-treated mice and hamsters were used in the Ames test, AAF mutagenicity was markedly suppressed.

The deleterious health effects experienced by primates exposed to low levels of polychlorinated biphenyls (PCBs) over an extended period of time are being determined. Infants born to female Rhesus monkeys which received diets containing 2.5 and 5.0 ppm PCBs for approximately 18 months were lighter at birth and had detectable levels of PCBs in their tissues. After nursing for two months, the infants began to display signs of PCB intoxication including acne, swelling of the eyelids and loss of eyelashes. Three of six infants died due to PCB intoxication. It was also determined that PCB levels in the milk ranged from 0.15 and 0.40 ppm. Upon weaning and placement on a synthetic milk replacement, these infants showed a decided improvement in their physical status. However, physiological defects appear to be present in these infants, such as, hyperactivity and unusual locomotor activity. The mothers, after having consumed a normal diet for one year, showed a decided improvement in their physical status. Their breeding performance was normal after this one-year period although the infants born to those exposed females continued to be small and showed detectable levels of PCBs in their tissues at birth. In addition, milk samples from these mothers range between 25 and 100 mg. PCBs per gram of whole milk, and offsprings continued to show abnormalities of thymus and lymphoid tissue as well as behavioral and learning deficiencies.

The affects of environmental pollutants on hepatobiliary-pancreatic function in animals are being studied to develop tests for the early detection of altered liver-pancreas function by these agents, and to determine the

mechanism by which the dysfunction is produced. It has been observed that chlorinated aliphatic hydrocarbons increase pancreatic fluid flow in rats, but the effect does not appear to involve secretin or cholinergic stimulation. Biliary excretion has been found to be the main excretory pathway for PCB and chlorinated biphenyl elimination. The literature reports that histological examinations of the pancreas from autopsy materials from animals exposed to chlorinated hydrocarbons, show no evidence of histopathology to the pancreas. However, on occasion, fatty degeneration of the pancreas and a typical pancreatitis from carbon tetrachloride exposure has been reported. The mechanism of this damage is being investigated.

Further clarification of the metabolic fate of PCB compounds is being carried out to determine the mechanisms by which the PCBs produced toxic changes in the mammalian tissues. Indications are that the PCB metabolites may, at least in part, be responsible for the lesions. An attempt is being made, therefore, to identify the metabolites that are present in the excreta and tissues of animals exposed to PCBs. A further objective is to determine the interaction of toxic metabolites with cellular macromolecules, thus permitting a clearer understanding of possible modes of action, and to evaluate these toxic metabolites in vitro and in vivo for further clarification of their necrogenic, carcinogenic and mutagenic potential.

An investigation is being made into the transport rates of foreign molecules into and across cell membranes and to attempt an understanding of their effects on the membranes, their structures and function. The foreign molecules of interest include: chlorinated hydrocarbons, anesthetics, metal alkyls and carcinogens. The effect of membrane composition on the foreign molecule-membrane interactions is also being investigated. In addition to precise chemical modification and analytical methods, the studies rely upon fluorescence and nuclear magnetic resonance spectroscopic techniques. An extensive survey of potential fluorescent probes for chlorinated hydrocarbons has identified carbazole as a fluorescent probe which is quenched by a wide variety of commonly-used chlorinated hydrocarbons. With the synthesis of carbazole-labeled phospholipids, a valuable new method to quantitate the amount of chlorinated hydrocarbons in cell membranes has resulted.

A study is being carried out to compare the solid state and solution confirmations of a number of physiological-active agents, including pyrethrin insecticides, Schiff's bases, and the drug, Trihexyphenidyl. In addition, the crystal structures of biologically active compounds with particular emphasis on potential anti-cancer agents are being determined. To date, the solution confirmations of several pyridoxal-amino-acid Schiff's bases have been established using NMR, kinetic and CAMSEO studies. A major accomplishment is the demonstration that a combined experimental-theoretical approach to solution confirmations and molecular pharmacology can provide more information than either one alone.

Benzene toxicity and metabolism studies are being carried out by measuring the effects of repetitive treatment of mice with ^3H -benzene. Toxicity is measured using the ^{59}Fe uptake technique. Benzene metabolites are being measured in urine, liver, spleen, fat and bone marrow. ^3H -benzene was given subcutaneously

to mice at doses of 450 mg/kg once or twice per day and 880 mg/kg, once or twice per day. At the lowest dose, iron uptake was reduced no more than 50 percent over a 20-day period. At a dose of 880 mg/kg, iron uptake was reduced to 80 percent of controls after 10 days and with 440 mg/kg twice a day, the iron uptake was reduced to 95 percent of controls over the same period. When 880 mg/kg was given twice per day, iron uptake was totally suppressed after three days. Urinary metabolites were measured, following the treatment and it was found that metabolism increased from days one through four, leveled off through day six and became progressively depressed through day ten. The initial effect may have been due to enzyme induction. During the same treatment schedule, it was found that free phenols and conjugates accumulated in blood, liver, fat and bone marrow. Conjugate levels were generally an order of magnitude greater than free phenols. Bone marrow contained the highest levels of both free phenols and water soluble metabolites. The studies have demonstrated that repetitive treatment with benzene produces gradually intensifying toxicity. There appears to be more conversion of benzene to metabolites early during treatment, perhaps, due to enzyme induction by benzene. The bone marrow contains high levels of benzene metabolites which appear to increase as benzene toxicity increases.

In another study, an attempt has been made to determine the biological significance and implication concerning the radicals and quinones of benzo(a)pyrene obtained through metabolism, and the damage of the reactive reduced oxygen species generated by this quinone, in a cyclic manner. The research has taken three directions, including an investigation of the metabolism of benzo(a)pyrene by the isolated nuclei and nuclear membrane, the toxicity of the quinone to the cells, the breakage of the cellular DNA by the quinone and by radiation. In addition, a special effort has been made to develop a sensitive method for the detection of DNA protein-crosslink which would be an indication of the attack by the radicals. The investigation, so far, has demonstrated that benzo(a)pyrene can be metabolized at the nuclear membrane in close proximity to the genetic apparatus. The nuclear membrane shows a preference for metabolism at the sixth position of benzo(a)pyrene to form a mutagenic compound 6-OH-BP. This phenol combined with DNA while undergoing autoxidation to BP diones through the 6-OXO-BP radical with the concomitant production of reduced oxygen species. The operation of the BP dione-BP-diol redox cycle could also generate reactive, reduced oxygen-species, as well as semi-quinone radicals very close to the genetic apparatus. These considerations may be very important to the mechanism of BP carcinogenesis.

A project is underway to define in humans and in animals the characteristics of the distribution, excretion and metabolism of the organochlorine pesticide, chloredecone (Kepone R). The information will be used as a basis for developing therapy for humans with overtotoxicity due to the exposure to large quantities of chloredecone and to estimate the hazard to asymptomatic humans with small amounts of chloredecone in the body. The findings of this group have already established that cholestyramine is a safe and practical therapy for humans with high levels of chloredecone in the body. It is felt that this therapeutic approach might be useful for treatment of poisoning with other lipophilic environmental toxins. The results of a five-month double blind controlled trial of cholestyramine in patients has been completed with the results that are both gratifying and exciting. In 22 patients, chloredecone

was eliminated slowly from blood ($T_{1/2}$ =165 days $\pm$ 27, SEM) and fat ($T_{1/2}$ =125 days, range 97-177) chiefly in the stool. Cholestyramine significantly accelerated the elimination of chlordecone from blood ($T_{1/2}$ =80 days $\pm$ 4, SEM, p 0.005) and fat ($T_{1/2}$ =64 days, range 52 to 95, p 0.05) compared to placebo-treated controls. The chemical detoxification was accompanied by a remarkable improvement in all aspects of the toxic manifestations of chlordecone.

The degradation of organic pollutants in the environment is being studied by a number of investigators. One such investigation involves the study of the rate and extent of PCB degradation by natural mixed populations of aquatic bacteria *in situ*, in order that the persistence of PCB in aquatic environments may be predicted. Such studies are expected to contribute to the understanding of the ecological effects and potential environmental health hazards posed by chronic or acute PCB contamination and accumulation of PCB or its metabolites.

The data obtained thus far indicate that PCB degradation can and does occur in the natural environment and that *in vitro* batch culture degradation studies are indicative that naturally occurring processes do occur. It is apparent that the endogenous population of bacteria found in moderately pristine waters can actively participate in PCB decomposition. The extent to which microbial populations degrade PCB in polluted or contaminated aquatic environments remains to be determined, as does the effects of the contaminants on the microbial community and the production of potentially-toxic metabolites of the pollutant.

An aerobic sporeformer has been isolated from pasteurized soil that is capable of growing on 4-hydroxyphenoxyacetate as a sole source of carbon and energy. This represents the first confirmation of the existence of a *p*-hydroquinone 1,2-dioxygenase, which was originally reported in 1965. Five strains of *Bacillus megatherium* and two strains of *Streptomyces* have been shown to be capable of converting Vanillate to Guaiacol in quantitative yields. The physiological function of this decarboxylation is yet to be determined. Guaiacol is not normally considered to be a natural product, but as an industrial by-product of such industries as the pulp and paper industry. Vanillate, on the other hand, is easily isolated from natural environments, such as in decomposing wood. The observation of microbial decarboxylation of Vanillate to Guaiacol shows that Guaiacol is a naturally occurring compound. These studies have further implications for the degradation of organic pollutants in the environment.

Enzymatic oxygen fixation is of physiological importance to all forms of life. Liver cytochrome P-450 catalyzes the mono-oxygenation of a variety of foreign compounds. In many instances, enzymatic oxygen fixation leads to the formation of electrophilic intermediates that have been implicated as the causative agent of chemical carcinogenesis. In spite of intensive investigation, the mechanism of oxygen fixation of mammalian enzymes remains to be elucidated. In one study, it has been shown that bacteria contain enzyme systems that incorporate both atoms and molecular oxygen into aromatic hydrocarbons and related compounds. In this respect, the bacterial enzymes are different from the mammalian enzymes, but no less important since these enzymes

initiate reaction sequences that result in the biodegradation of many environmental pollutants and carcinogens. Elucidation of the mechanism of action of oxygenases could have important bearing on human health and environmental quality.

A study of the metabolism, disposition and interaction of water-born pollutants in fish is being supported to determine the factors governing persistence of residues in fish destined for human consumption, and the extent and identity of metabolites of selected hazardous compounds in fish. Carbaryl (Sevin) significantly increased the acute toxicity of a number of pesticides and herbicides, such as, rotenone, 2,4-D, n-butylester, dieldrin, pentachlorophenol and Bayer 73. Mechanistic studies indicated that carbaryl may increase acute toxicity by increasing uptake of these agents from water by the trout. Studies of di-2-ethylhexylphthalate (DEHP) indicated 50 percent concentration of parent compound and metabolites in the bile. The major metabolite was identified, and accounted for 72 percent of the total bile concentrated. Polychlorinated biphenyls (PCBs) were also studied for tissue distribution and metabolism. Enzyme-inducing agents, such as, phenobarbital and polycyclic aromatic hydrocarbons were used to alter this metabolism and the observed effects are currently under analysis. These studies will serve to develop predictive capability concerning hazardous materials entering the aquatic environment and will assist regulatory agencies in assuring a safe water and protein supply for man.

NATURAL TOXINS

The hemophilic activity and in vivo effects on various ATPase activities of jellyfish toxin is under investigation. Initial studies have shown that calcium ion is essential for hemophilic activity, although at high concentrations (greater than 26 millimole calcium) hemolysis is inhibited. The hemophilic activity was lost if the toxin remained in solution at 4 degrees for 72 hours, and the protein was denatured at pH greater than 9 or below 4. The hemophilic factor has now been isolated in homogeneous form and characterization of this component is underway. The in vivo ATPase activities have been determined in various tissues of mice (hearts, livers, kidneys and brains) and the data from these experiments are currently being analyzed.

It has been established that aflatoxin and other mycotoxins are present in human and animal foodstuffs. The effects of these mycotoxins on the embryo (embryocidal, teratogenic), are being determined. Citrinin and viriditoxin (toxic fungal metabolites) were administered to pregnant mice with no deleterious consequences. Both penicillic acid and penitrem A were found to cause skeletal defects in offsprings from treated mothers. Reduced fetal weights were observed at the high doses of penicillic acid. Interaction of aflatoxin B₁, ochratoxin A and rubratoxin B on infant rats demonstrated no synergistic toxicity. The mutagenic effects of a series of mycotoxins on Salmonella typhimurium was determined and only aflatoxin B₁ and sterigmatocystin were mutagenic.

To gain an understanding of the mechanism by which the toxins from the Portuguese Man-of-War affects the cardiovascular system of animals, purified

toxin is being utilized on whole perfused heart preparations and on isolated nerve/muscle preparations. The effects of this toxin on active sodium transport and its effect on various ATPases activities are also under investigation. A series of cause-effect relationships is being sought. It has been demonstrated by using red blood cells as a source of ATPase that low-toxin concentrations cause slight activation of the calcium ATPase, while at higher concentrations, the inhibition becomes linear. The toxin also induced well-defined spontaneous contractions in isolated frog gastrocnemius preparation, although it did inhibit the response of these muscles to externally applied electrical stimuli. In addition to these smooth muscle and skeletal muscle observations, the toxin has been found to have direct contractile effect on myocardial vasculature. With an isolated rat heart, approximately 30 micrograms of toxin produced immediate tachycardia followed by a prolonged period of bradycardia. A 10-fold increase in dose, caused a brief series of rapid beats followed by immediate arrest in systole. Finally, it was determined that atropine is very effective in preventing the arrhythmias produced by the toxin in the isolated heart preparation. However, the drug does not block the vasoconstrictive action of the toxin on the coronary vasculature. These findings may provide the basis for medical treatment of individuals suffering from severe stings.

A material provisionally called aponin, produced by the blue-green algae Gomposphaeria aponina, that is cytolytic toward the red tide organism Gymnodinium breve has been found and is undergoing evaluation. The inorganic nutrient requirement of G. aponina has been characterized and the physiological limitations determining the production of aponin have been completed. The biological interactions of G. aponina and the cytolytic factor, aponin, on ecologically important organisms are being observed. No significant effects were found on two selected species of fish at concentrations from 0 to 15 aponin units. In addition, when low levels of aponin were mixed with G. breve cultures, the mean survival of fish in these cultures was extended. However, at higher aponin levels, survival-time was reduced. With crustaceans (brine shrimp) no short-term deleterious effects were found due to aponin alone. In mixed cultures, survival times were increased and higher doses of aponin were found to be deleterious. Efforts are underway to chemically characterize aponin and to prepare pseudo-aponin chemically. The success of these endeavors may result in a safe methodology for controlling red tide outbreaks.

The modes of action of neurotoxins from C. sculpturatus venom are under investigation to establish appropriate neurotoxins as future pharmacologic reagents to be used as tools to elucidate basic physiological and toxicological principles and mechanisms of action. Neuromuscular studies of crude venom action were conducted with standard intracellular techniques on the frog neuromuscular preparation. Neurophysiological data have been accumulated and repetitive neurofiring has been observed. Materials from venoms of other species were compared to further define properties of these useful physiological agents. Information on these venoms may lead to insight toward appropriate treatment for individuals subjected to the venoms through stings or bites.

Detailed biochemical studies of naturally occurring food toxicants, such as toxins from mold-damaged sweet potatoes and other toxic plants continue to produce interesting results. Structure-activity relationships of several congeners of perilla ketone and 4-ipomeanol indicated characteristics which increased or decreased the lung toxicities of these compounds. Efforts at purifying, chemically characterizing, and identifying other mold-produced food toxins are in progress.

The lethal and pain-producing factors present in sea nettle venom are being defined and isolated. A radioimmunological assay system for bradykinins, and Sephadex separation has been used to identify and isolate the lethal factors from the venom. Allergen specific immunoglobulin E concentrations were found to vary significantly when the venom was exposed to patient's sera. These results indicate the capability of these materials to induce an allergic state in humans and the possible utility of this system as a screening device to detect susceptible persons.

Our understanding of the mode of action of the synergistic neurotoxicity of the components of Crotoxin has been advanced along several lines. Chemical modification studies have shown that Crotoxin B, which is by itself phospholipase A₂-active, but not neurotoxic, loses both activities when one of its two histidines (not the N-terminal one) becomes specifically alkylated by p-bromophenacyl bromide. This specific affinity is characteristic of many, if not all, phospholipase A₂'s. However, in the Crotoxin complex, which is both enzymatically and neurotoxically active, none of the three histidines react. Blocking of amino groups by acid anhydrides which attack no other groups in these proteins, progressively inactivates both Crotoxin B and the complex; in A, only one of three amino groups becomes alkylated and activity is not lost. Thus, it is concluded that amino groups are not part of the enzymatically active site but cooperatively contribute confirmational stability to the enzyme which is essential for its action. The Crotoxin complex was found to be able to block the response of electroplaques of the electric eel (Electrophorus electricus) to carbamyl choline similar to that observed with alpha-bungarotoxin. Crotoxin B showed the same activity but Crotoxin A was found inactive. Whether the seemingly postsynaptic effects of Crotoxin are of neurophysiological importance remains uncertain. Another study has been concerned with the relationship of neurotoxicity and phospholipase A₂ activity.

Although it has been established that ochratoxin A is a nephrotoxic compound, little work has been done to characterize biochemically the effect of this toxin in the kidney. A project is underway to define the mechanism for the action of ochratoxin A at both the cellular and sub-cellular levels. The initial experiments were designed to examine the hepatotoxic and nephrotoxic effects of ochratoxin A on an acute basis. This was accomplished by injecting rats intraperitoneally with ochratoxin A and subsequently measuring glycogen phosphorylase activity in glycogen levels at intervals, in the liver and the kidney. The results of the study suggest that ochratoxin A is causing a perturbation of the glycogen phosphorylase system. This attempt to understand how a particular mycotoxin causes its pathological symptoms is continuing.

GENETICS

OFFICE OF THE ASSOCIATE DIRECTOR FOR GENETICS
Summary Statement

During the past year the Office of the Associate Director for Genetics has continued old programs and developed new projects in the area of environmental mutagenesis in the following areas (1) International Programs (2) National Programs (3) Training Courses (4) Committees (5) Collaborative Studies (6) Collaborative Research Programs, and (7) Public Lectures.

International Programs

A series of meetings of the International Commission for Protection Against Environmental Mutagens and Carcinogens (ICPEMC) have been attended by the Associate Director for Genetics who serves as the Vice-Chairman. The second meeting of the Executive Committee and Commission was held in Lausanne, Switzerland on September 26-28, 1977. The third meeting of the Executive Committee was held at NIEHS on February 27-28, 1978. The fourth and fifth meetings of the Executive Committee and Commission were held in Lausanne on April 9-13, 1978 and September 8-13, 1978, respectively. These meetings consisted of developing the administrative framework of ICPEMC as well as defining, initiating and evaluating the work of various task forces and subcommittees.

Under the US-Japan Cooperative Medical Sciences Program, the joint environmental Panels organized a Sixth Joint Conference on "Modification of Mutagenic and Carcinogenic Activity" which was held October 24-26, 1977 in Williamsburg, Virginia. A wide range of factors were discussed including genetic, biochemical and environmental. It was clear from the presentations that mutagenic response can be modified markedly both quantitatively as well as qualitatively.

The Seventh Joint Conference held under this program was on the "Utility of Sister-Chromatid Exchange" which was held on July 16-17, 1978 at Lake Yamanaka-Ko, Japan. Following this workshop a 5-year review of the work of the US and Japanese Panels was held in Tokyo on July 18-19, 1978.

Under the US-USSR Environmental Protection Agreement, the Fifth Joint Conference on Basic and Practical Approaches to Environmental Mutagenesis and Carcinogenesis was held on May 30 - June 1, 1978 in Baku, Azerbaijan, S.S.R. The program discussed development and validation of new techniques for screening environmental chemicals. These assays included in situ assay systems such as ferns, inhibition of DNA synthesis, in vitro transformation, use of repair-deficient strains and the use of various bacteria with in vitro metabolic activation. Following the workshop the US delegation visited the new laboratory facilities of the Institute of General Genetics in the Puschino Region outside of Moscow.

National Programs

A workshop on Comparative Chemical Mutagenesis organized by the Associate Director for Genetics was held in Raleigh, NC on October 30 - November 4, 1977. The main objective of this workshop was to compare data on the mutagenicity of 20 mutagens which have been studied extensively for the genetic effects. Both quantitative and qualitative effects were compared over a wide range of assay systems. In a series of papers, the genetic effects of these chemicals were reviewed as a function of assay systems and then these reports were used to summarize the effects as a function of chemical. The workshop was international in scope with over 125 participants. Position papers were prepared by leading research groups in the area of environmental mutagenesis from all over the world. Individual position papers have been prepared for distribution through EMIC and will eventually be collected in the form of a book.

A workshop to explore "The Utility of Higher Plant Systems as Monitors of Environmental Pollutants" was held in Marineland, Florida on January 16-18, 1978. The workshop discussed various plant genetic systems which have been applied in environmental mutagenesis studies. Approaches included:

1. Sample collection and concentration with assays for mutagenicity on eluted fractions with a laboratory based assay using Salmonella or other microorganisms.
2. Using naturally occurring organisms which accumulate chemicals from the ecosystem and testing extracts of these organisms with laboratory based assay systems.
3. Measuring genetic damage in selected populations of organisms naturally occurring in a given area.
4. Introducing an assay system into a polluted environment for a short period and then determining the changes in mutation rate.

Training Courses in Environmental Mutagenesis

This office has participated in the development of a 3-day training course for administrative personnel sponsored jointly by the Environmental Mutagen Society and the University of Texas Medical School, Galveston, which was held on May 23-25, 1978 in Hunt Valley, Maryland.

Committees

Subcommittee on Environmental Mutagenesis monthly meetings chaired by the Associate Director for Genetics have been held to review current problems in environmental mutagenesis; a detailed list of meetings and minutes have been submitted to the parent Committee (DHEW Committee to Coordinate Toxicology and Related Programs).

During FY 78 the Associate Director has also participated as a consultant to the EPA Environmental Health Advisory Committee in the development of criteria and guidelines for mutagenicity testing of pesticides. In addition he has also worked as a member of an FDA task group to develop criteria and guidelines for mutagenicity testing of food additives with special emphasis on GRAS list chemicals.

Collaborative Studies

The MRC/ICI/NIEHS International Trial to test paired compounds consisting of chemical carcinogens and noncarcinogenic structural analogs as well as selected chemicals to serve as positive and negative controls has been continued. This study has been developed in cooperation with the Medical Research Council and the Imperial Chemical Industries in Great Britain, the International Association of Environmental Mutagen Societies and NIEHS. The study will involve collaboration between 50 scientists in different laboratories all over the world who will compare various assays for mutagenicity in blind tests on about 42 compounds.

Collaborative Research Programs

The Associate Director for Genetics has continued the analysis of the mutagenic activity of various classes of chemical carcinogens in wild-type and excision-repair deficient two-component heterokaryons of Neurospora crassa. This work is being performed with the collaboration of the University of Illinois, Normal, Illinois and the Miles Laboratory, Elkhart, Indiana (see LEM report). These studies are designed to determine whether chemicals are carcinogens because they produce some characteristic mutagenic lesion. Previous studies have shown that in wild-type *Neurospora*, chemical carcinogens are base-pair substitution mutagens, whereas in excision-repair deficient *Salmonella* they are frame-shift mutagens. Current data in *Neurospora* where comparisons have been made between wild-type and excision-repair deficient strains shows that base-pair substitution mutagens in wild-type strains are converted to frame shift mutagens in excision-repair deficient strains. More extensive data are being sought by testing chemical carcinogens from different chemical classes to determine the generality of these conclusions.

In another collaborative research program, a higher plant system (*Tradescantia*) is being developed and used in a mobile laboratory to monitor airborne pollutants in collaboration with the Brookhaven National Laboratory (BNL) and ERC-EPA. Plans have been made to monitor the ambient air in various high cancer incidence counties around the country during the next year. Significant increases in mutation frequencies have been found in six of the eight cities monitored during the first half of FY 78. These are exploratory studies to evaluate the general utility of this assay system as well as to determine the type of air pollutants that will give a positive response with this assay system. The group at ERC, EPA has the responsibility for providing data for selection of sites as well as providing the physical monitoring data concerning the spectrum of pollutants

(nonorganic and organic) in ambient air at each site. The preparation of plant material and evaluation of mutagenic activity is carried out at BNL.

In another area attempts are being made to evaluate the ability of an assay system which has been developed in Neurospora crassa to screen chemicals for nondisjunction. Significant progress has been made in both validating this procedure as well as evaluating its general utility as a prescreen.

Public Lectures

F. J. de Serres

1. "Introduction: Training and Education in the Field of Environmental Mutagenesis", Second International Conference on Environmental Mutagens, Edinburgh, July 11-15, 1977.
2. "Current Strategies in Environmental Mutagenesis", Environmental Health Center, Environmental Toxicology Division, Ottawa, Canada, September 13, 1978.
3. "Genetic Modification of the Mutagenic Activity of Chemical Mutagens and Carcinogens in Neurospora crassa", Workshop on Modification of Mutagenic Activity, Williamsburg, VA, October 24-26, 1977.
4. "Introduction: The Utilization of Higher Plant Systems as Monitors of Environmental Mutagens", Workshop on Higher Plant Systems as Monitors of Environmental Mutagens, Marineland, Florida, January 16-18, 1978.
5. "Higher Plant Systems as Monitors of Environmental Mutagens", Symposium on Application of Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures, Williamsburg, VA, February 21-23, 1978.
6. "Introduction: In Vitro Tests for Identifying Environmental Carcinogens", 31st Annual Symposium on Fundamental Cancer Research, Houston, Texas, May 1-3, 1978.
7. "Comparison of the Mutagenic Effects of Chemical Mutagens in Excision-Repair Deficient and Wild-Type Two-Component Heterokaryons of Neurospora crassa," Ninth Annual Meeting of the Environmental Mutagen Society, San Francisco, CA, March 9-13, 1978.
8. "Comparison of Carcinogen-Induced Mutation in Wild Type and Excision-Repair Deficient Strains of Neurospora", National Institute of Environmental Health Sciences, Research Triangle Park, NC, March 15, 1978.
9. "Human Mutations and Toxic Chemicals in our Environment", Department of Human Genetics, Medical College of Virginia, Richmond, VA, March 16, 1978.

10. "Monitoring for Chemical Mutagens", 3rd Annual Symposium on Genetics for the Practicing Physician, New York, NY, May 7, 1978.
11. "Microbial Test Systems", Workshop on Methodology for Assessing Reproductive Hazards in the Work Place, Bethesda, MD, April 19-23, 1978.
12. "Environmental Hazards and the Application of Mutagenic Tests", 26th Annual Meeting Radiation Research Society, Toronto, Canada, May 14-18, 1978.
13. "Risk Estimates from Animal Data", First Industrial Workshop in Chemical Mutagens; Principles and Methods of Detection, Hunt Valley, MD, May 22-25, 1978.
14. "Evaluation of Tests for Mutagenicity as Indicators of Environmental Carcinogens", Symposium on Public Control of Environmental Disease, New York Academy of Sciences, June 28-30, 1978.

Michael D. Shelby

1. "The Environmental Mutagen Information Center", Workshop on Higher Plant Systems as Monitors of Environmental Mutagens, Marineland, Florida, January 16-18, 1978.
2. "Short-Term Prescreening Tests - Present Position at NIEHS and International Collaboration", Toxicology Forum, Washington, DC, February 19-22, 1978.
3. "Potential of Plants as in situ Monitors for Environmental Mutagens", Symposium on Monitoring Biological Effects of the Environment, Raleigh, NC, March 22-23, 1978.

OFFICE OF HEALTH HAZARD ASSESSMENT

OFFICE OF HEALTH HAZARD ASSESSMENT
Summary Statement

This office, composed of six professionals and support personnel, is concerned with the evaluation of human health hazards due to physical or chemical factors. It has been realized that many chemical as well as physical agents may be responsible for long-term toxicity, particularly some of the dreaded diseases like cancer, neurotoxicity, and genetic defects. It has thus become an order of high priority to search for such toxic agents, to become aware of their potential disastrous effects, and to protect the public. However, the problem is not simply the detection of hazardous chemicals, etc., but to determine the hazard in a quantitative manner, to be able to make correct health hazard assessments. For all hazardous chemicals there are no effect levels; but for those that accumulate in biological systems and those that interact with other environmental factors to either enhance or possibly decrease the adverse effect, much more consideration has to be given to these possibilities.

One of the tasks involving all professionals in this office is to compile a reference document which will bring together literature data on disease and symptoms of disease for each organ and tissue with exposure data to potentially etiologically connected chemicals. To confirm the causative nature of the association, the literature is searched for results of laboratory studies which would establish such cause and effect relationships.

Another major task is to bring together all experimental evidence of biochemical mechanisms at work in mammalian systems which could prevent or alter the outcome of exposure to toxic chemicals. These considerations deal with the multiple pathways of metabolism of chemicals leading to derivatives that could be more or less active in expression of toxicity than the parent substance, as well as following metabolism further, to detoxification and final elimination. There may be many interacting factors at work opening or closing important pathways, but above all there is the evidence that every metabolic pathway can be overloaded by increasing the dose of the chemical administered. This factor needs the greatest attention for proper extrapolation of data. There are many other biochemical mechanisms at work involving DNA repair: enzyme systems of various types which have different characteristics in different species, organs, and affect different alkylated sites on DNA. There are immunological factors at work which may be altered at will. All these physiological and biochemical modifiers must be considered in appropriate health hazard assessment.

Since the Toxic Substances Control Act was passed by Congress, this Institute has supplied a member and alternate (Dr. Falk and Dr. Piver) to the Inter-agency Committee for Selection of Substances for Testing in order to help EPA in carrying out this mandate. The activity of the Interagency Committee is a steadily on-going activity searching for the chemicals most in need of further testing.

Dr. Damstra is the NIEHS representative on the Interagency Toxic Substances Data Committee (ITSDC), formed to design and coordinate an effective system for the retrieval of information on chemical substances submitted to EPA under the Toxic Substances Control Act. Dr. Damstra chairs a Chemical Substances Information Network Subcommittee of the ITSDC which will address how a wide variety of data bases, containing data pertaining to toxic chemicals, could be integrated into one information network.

Dr. Damstra is also the NIEHS representative on the Toxicology Information Subcommittee (TIS) of the DHEW Committee to Coordinate Toxicology and Related Programs. This committee identifies the needs and establishes the mechanisms for the collection, storage, and dissemination of toxicologic information within DHEW. Dr. Damstra also serves as a member on the TIS Task Group for the Laboratory Animal Data Bank (LADB). LADB is an on-line computer system providing comparative clinical/pathological data on laboratory animals and containing descriptive information on various animal strains.

Collaboration with the World Health Organization of which this Institute is a collaborating center has continued this fiscal year involving staff of this office. Dr. Piver continued to be involved with the preparation of a WHO-sponsored document on "Environmental Health Problems Associated with the Manufacture of Synthetic Organic Chemicals". The Director, OHHA, participated in a meeting May 1 to May 5, 1978, at Geneva, to help implement World Health Assembly Resolution 30.47 on a new approach regarding the international evaluation of the effects of chemicals on health. The Director, OHHA, also attended a meeting of WHO-EURO in Brussels on March 20-23, dealing with the health consequences of chemicals occurring naturally in drinking water. He also served as chairman of the scientific advisory committee to the International Register of Potentially Toxic Chemicals of the United Environmental Program in Geneva, December 5-9.

Staff of the OHHA continues to collaborate with other governmental agencies giving constructive criticism in the evaluation of Environmental Impact Statements, Effluent Limitation Guidelines, health criteria documents, and giving advice to the U.S. Department of Interior on Research Proposals under PL 88-379. These activities consume a good portion of the total available manpower-time.

Considerable efforts were made by staff of OHHA to contribute expertise to other agencies. Thus Dr. Posner was a member of the Working Group on National Ambient Air Quality Standard for Lead, while Dr. Martin reviewed the document for EPA. Dr. Falk participated on a committee evaluating the disposal of lethal chemical agents and similarly participated on the Committee for Human

Use regarding chemical demilitarization for the Department of Defense. Dr. Falk became a member of the International Joint Commission's Committee on the Assessment of Health Effects of Great Lakes Water Quality and completed his participation on the expert panel on Nitrites, Nitrates, and Nitrosamines for USDA.

For the Department of Energy, a Committee on Health and Ecological Effects of Increased Coal Utilization was set up and Drs. Falk and Jurgelski were asked to write a background paper on "Carcinogens and Cofactors" for this committee. Dr. Falk presented the contents of the paper at an open meeting.

Work on committees inside the DHEW was carried out by Dr. Damstra with regard to environmental chemical crises; by Dr. Piver regarding the review of the hazardous waste management resource recovery act as well as on the design of efficient incinerators for hazardous waste disposal; by Dr. Posner attending the first Annual NIOSH Scientific Symposium; and by Dr. Falk as member of the Committee on Safety Standards for Laboratory Operations Involving Chemical Carcinogens.

For NIEHS Dr. Piver participated in the deliberation of the Program Planning Group based on the second task force report on Human Health and the Environment -- Some Research Needs. Dr. Falk served on the NIEHS Committee on Smoking and the NIEHS Chemical Selection Committee of the Environmental Mutagenesis Test Development Program. Dr. Damstra was chairman and speaker at the NIEHS-sponsored Target Organ Conference on October 19-21 on the topic of neurochemistry and toxicologic overview.

Staff members also participated in other meetings as invited participants or speakers. Dr. Falk gave an invited lecture on Environmental Carcinogenesis to the American Association of Clinical Chemists in San Francisco. Dr. Posner gave lectures on Prenatal Maldevelopment at Wayne State University, and a paper at the Association of Food and Drug Officials meeting in Indianapolis on the topic, "Environmental Contaminants". He also became Section Secretary on Toxicology for the North Carolina Academy of Sciences and acted as session chairman at their first meeting on toxicology.

Dr. Piver attended a conference on Health and Environmental Effects of Oil Shale Technology in Denver, and Dr. Jurgelski participated in the 17th Hanford Biology Symposium on Developmental Toxicology of Energy Related Pollutants. He also attended the 31st Annual Symposium on Fundamental Cancer Research at Houston, Texas, which dealt with "Carcinogens: Identification and Mechanisms of Action". He will give a paper at the 8th International Congress of Neuro-pathology on "Chemical Induced Gangliogliomas in the Opossum" in Washington, D.C.

Dr. Damstra participated in the Behavioral Teratology Workshop at Macinaw Island and acted as judge at the North Carolina Student Academy of Sciences competition. She was also recognized as "Woman of the Year" by the Eno Business and Professional Women's Club of Durham.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 20001-06 OHHA
PERIOD COVERED October 1, 1977, to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Variations in the Response of Rats to Porphyrigenic Chemicals		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Hans L. Falk OTHER: Sandra Jordan Assoc. Dir., OHHA Technician OHHA NIEHS OHHA NIEHS		
COOPERATING UNITS (if any) None		
LAB/BRANCH None		
SECTION None		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, N.C. 27709		
TOTAL MANYEARS: 1.1	PROFESSIONAL: 0.1	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline ! Studies have shown that individual rats of the same strain and sex vary in their response to standard doses of porphyrigenic chemicals. This variation is shown in the time it takes to respond by porphyrinuria, the time for porphyrin excretion to return to normal levels, and the intensity of the response. Some animals were found not to respond at all even after repeated doses of different porphyrigenic chemicals. Hexachlorobenzene (HCB) was the primary chemical on study because of its importance as a fungicide. Allyl isopropylacetamide (AIA) was used to study acute responses to rapid-acting porphyrogens. The response to AIA also varied among individual animals.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Female C.D. rats were given standard doses of HCB in the diet for short time intervals. AIA was administered by gavage in cottonseed oil, given in the food, or by subcutaneous injection. Uro- and coproporphyrins were extracted from the urine and quantitated spectrophotometrically. Animals were checked at regular intervals for porphyrin excretion. Gross fluorescence was looked for in feces, and tissues and organs following necropsy. Control animals were checked at similar intervals for porphyrinuria.

MAJOR FINDINGS AND PROPOSED COURSE: Urinary excretion of uro- and coproporphyrins in rats fed hexachlorobenzene in the diet varied from no response to a level 12 times higher than normal porphyrins in the urine. Maximum response was observed eight to nine months after HCB administration. It declined slowly to normal levels after 18 months. There is apparently a difference in response depending on the age of the animals when HCB feeding is started. Weanling rats (24 days old) seem to take longer to reach peak excretion levels of porphyrins and take longer to return to normal levels when compared to animals started at an older age (60 days).

Studies will be carried out with radioisotope-labelled delta-aminolevulinic acid injected into normal, porphyric and non-responding rats to trace utilization of delta-ALA in porphyrin and heme biosynthesis. Rates of excretion will be followed by urinary and biliary routes. Studies will also be conducted by administration of radioisotope-labelled HCB to weanling and older rats to check for differences in absorption, metabolism and storage of this porphyrogen.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Variations in human response to environmental chemicals are always observed but such drastic variations have not been followed in laboratory studies to any extent. The very marked variation in the response to porphyrogenic chemicals may serve as a model to study differences in toxicologic responses and to determine the factors responsible for them.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 20002-06 OHHA																
PERIOD COVERED October 1, 1977, to September 30, 1978																		
TITLE OF PROJECT (80 characters or less) Technology Forecasting and Technology Assessment																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">Warren T. Piver</td> <td style="width: 35%;">Chemical Engineer</td> <td style="width: 15%;">OHHA NIEHS</td> </tr> <tr> <td>OTHER:</td> <td>Hans L. Falk</td> <td>Assoc. Dir. for OHHA</td> <td>OHHA NIEHS</td> </tr> <tr> <td></td> <td>Herbert S. Posner</td> <td>Pharmacologist</td> <td>OHHA NIEHS</td> </tr> <tr> <td></td> <td>Terri Damstra</td> <td>Biochemist</td> <td>OHHA NIEHS</td> </tr> </table>			PI:	Warren T. Piver	Chemical Engineer	OHHA NIEHS	OTHER:	Hans L. Falk	Assoc. Dir. for OHHA	OHHA NIEHS		Herbert S. Posner	Pharmacologist	OHHA NIEHS		Terri Damstra	Biochemist	OHHA NIEHS
PI:	Warren T. Piver	Chemical Engineer	OHHA NIEHS															
OTHER:	Hans L. Falk	Assoc. Dir. for OHHA	OHHA NIEHS															
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SUMMARY OF WORK (200 words or less - underline +) The goals of this program are: (1) develop and apply techniques for technology forecasting and technology assessment for the chemical process industries and the energy production industries that would provide guidance in setting research priorities in environmental chemicals from these two sources and (2) develop environmental transport models and methods of parameter estimation for use in designing solid and liquid waste management practices for chemicals from these two sources.																		

PROJECT DESCRIPTION

METHODS EMPLOYED: For both objectives, computer simulation is the main research tool. Trends in production, rates of substitution of products, and the impact of Federal legislation and programs on chemical and industrial developments can be modeled. This information can be coupled with available basic physical-chemical, biomedical and toxicological data to provide preliminary environmental assessments and point out gaps in information.

MAJOR FINDINGS AND PROPOSED COURSE: As a result of Federal legislation, a large number of chemicals are being developed and marketed as fire retardant chemicals. In many instances the mechanisms of retardation and the nature of the combustion products are poorly understood. In addition, the toxicities of the chemical and its combustion products are poorly characterized. Federal energy programs have called for the development of large deposits of coal and oil shale for energy production. The environmental health impacts of this type of development are poorly understood, particularly in the area of management of solid and liquid wastes from these processes. Efforts are currently underway to simulate the transport in solid waste disposal sites for the purpose of developing better waste management practices. This information and approach can be used in other solid waste management programs. In addition, an extensive effort is underway to examine the synthesis of industrial organic chemicals. This allows the connection of the organic and inorganic chemical industrials, and identifies hazardous materials that are contaminants and by-products and relates them to the synthesis of the chemical. The coupling of possible relationships between chemical structure and biological activity, with a forecast of the rate of substitution of chemical products in the market place, is useful in determining the types of biomedical research programs needed to assess the environmental health impact of commercialization of new products.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These activities will continue to provide necessary information about products and use patterns of chemicals, important environmental transport mechanisms, and development of methods to predict exposure levels for environmental chemicals. Such information can be used in planning research programs for the Institute.

PROJECT DESCRIPTION

METHODS EMPLOYED: Searching of the literature, consultations with appropriate individuals, consideration of structure-activity relationships, preparation of reports, discussions with those who might assist in further laboratory or theoretical considerations, feedback and dissemination of the appropriate findings, and preparation of manuscripts for publication; reviews of documents and manuscripts and response to letters as requested.

MAJOR FINDINGS AND PROPOSED COURSE:

Aminimide compounds: This interesting and relatively new series of chemicals is being monitored in the scientific literature. The broad spectrum of antimicrobial activity previously reported was studied further with a large number of acylaminimides. Comparison of structure and reactions is periodically reviewed in the literature for these $\ddot{N}-\ddot{N}$ -species and other ylide compounds. Ylides are dipolar compounds in which any of a variety of adjacent positively and negatively charged atoms are covalently linked.

Compounds with a vinyl halide substructure: A list of compounds containing a vinyl halide group was prepared for presentation in May 1977 at the NIEHS Conference on Comparative Metabolism and Toxicity of Vinyl Chloride Related Compounds. The list was considerably enlarged and further sorted by structure. With few exceptions only compounds were included that have been used commercially, or are either present as contaminants in other compounds or are present in nature. Two hundred and sixty-two compounds were presented with their names and uses. Although the search was broad, it was not exhaustive. Some classes of compounds were omitted and the reasons given. An attempt will be made at hazard analysis with some of the compounds and further compounds will be added as these are identified.

Methanol: Methanol is still occasionally mentioned for possible applications as an alternate vehicular fuel, alone or mixed with gasoline. Communications are being maintained with some appropriate individuals world-wide. It appears that ethanol is gaining in acceptance over methanol in Brazil and Australia. Furthermore, the process of catalytic conversion of methanol to a mixture of hydrocarbons similar to gasoline, developed in the United States, may be economically feasible in the future. Confinement of methanol to sealed system, large-scale applications would be less hazardous. If this were done, then the methanol would not be available to be inhaled, absorbed through the skin or to be drunk for its weak imbibing effect.

Lead: I participated in the meetings of the Working Group on a National Ambient Air Quality Standard for Lead and reviewed the documents produced. The comments were reviewed from an EPA hearing and final documents are being completed by EPA.

Dr. Terri Damstra and I were invited to combine and condense our Environmental Health Perspectives reports on lead hazard. Additional information was added by Dr. Jerome O. Nriagu of the Canada Centre for Inland Waters and the chapter will appear in the book "The Biogeochemistry of Lead in the Environment."

Dibromochloropropane (DBCP): The Director's office was notified and kept informed of news reports of the fast-breaking issue of low or absent sperm counts and sterility in DBCP industrial workers. References were provided to him of reports of experimental animal carcinogenesis due to DBCP and ethylene dibromide (EDB), and also to experimental mutagenesis with DBCP. We found preparations of 10-30% DBCP on sale in garden supply stores in Chapel Hill, Durham and Burlington, North Carolina despite a newspaper report that there was very limited home and garden use. The compound is approved for use on a large number of fruits, nuts, vegetables, field crops, and ornamentals. Inter-governmental review and prompt actions by the companies that were involved led to a rapid institution of control for the compound pending further information.

Stratospheric modification: The Federal Task Force on Inadvertent Modification of the Stratosphere (IMOS) and the Biological and Chemical Effects Research Subtask Force (BACER) completed their work and my activities ended. The BACER Policy Steering Committee was upgraded to the Stratospheric Protection Coordinating Committee in accord with the August 1977 Amendment to the Clean Air Act. Dr. David P. Rall, Director of NIEHS, is the Institute's representative to the Committee.

Card file of chemical and physical agents considered in NIEHS intramural research: A card file was prepared of the experimental chemicals and physical agents mentioned in the two previous NIEHS Annual Reports and agents listed in the current report will be added. The file allows us to identify all projects in which a particular agent was indicated as either considered or used.

Other NIEHS and outside activities: Documents from NIOSH, EPA, FDA, and CPSC were reviewed for their health hazard content. I presented invited talks to the International Fertility Research Program, the Area Health Education Committee of Wake County, the Central Carolina Chapter of the Professional Engineers of North Carolina, and the Association of Food and Drug Officials. I was the secretary of the newly established Toxicology Section of the North Carolina Academy of Sciences and Chairperson of a scientific session at its Spring meeting with the Academy.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The project identifies areas where research is needed in order to prevent or reduce toxicologic hazard from environmental chemicals or physical agents. It is primarily a program of preventive and early-warning surveillance. It is linked closely to research dealing with the scope of the problem, the mechanisms involved, and preventive measures. It also considers potential means of diagnosis and therapeutics where these are possible.

PUBLICATIONS

Posner, H. S. and Falk, H. L.: Haloethylene-related compounds of industrial, environmental, and medical significance. Environ. Hlth. Perspec. 21: 293-326, 1977.

Posner, H. S.: Indices of potential lead hazard. Environ. Hlth. Perspec. 19: 261-284, 1977.

Posner, H. S.: The recommendations of the Task Force on Inadvertent Modification of the Stratosphere. In Spraydose Verboten? (A Ban on Aerosols?) Rüslikon-Zürich. Gottlieb Duttweiler Institute for Economic and Social Studies, 1977, pp. 319-328.

Posner, H. S.: Letter to Editor. CHEMTECH 196, April 1978.

Posner, H. S., Damstra, T., and Nriagu, J. O.: Human health effects of lead. In Nriagu, J. O. (Ed.): The Biogeochemistry of Lead in the Environment. Amsterdam, Elsevier (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 20004-04 OHHA																
PERIOD COVERED October 1, 1977, to September 30, 1978																		
TITLE OF PROJECT (80 characters or less) Surveillance of Potential Environmental Health Hazards																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																		
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 40%;">Terri Damstra</td> <td style="width: 25%;">Biochemist</td> <td style="width: 20%;">OHHA NIEHS</td> </tr> <tr> <td>OTHER:</td> <td>Hans L. Falk</td> <td>Assoc. Dir. for OHHA</td> <td>OHHA NIEHS</td> </tr> <tr> <td></td> <td>Warren T. Piver</td> <td>Chemical Engineer</td> <td>OHHA NIEHS</td> </tr> <tr> <td></td> <td>Herbert S. Posner</td> <td>Pharmacologist</td> <td>OHHA NIEHS</td> </tr> </table>			PI:	Terri Damstra	Biochemist	OHHA NIEHS	OTHER:	Hans L. Falk	Assoc. Dir. for OHHA	OHHA NIEHS		Warren T. Piver	Chemical Engineer	OHHA NIEHS		Herbert S. Posner	Pharmacologist	OHHA NIEHS
PI:	Terri Damstra	Biochemist	OHHA NIEHS															
OTHER:	Hans L. Falk	Assoc. Dir. for OHHA	OHHA NIEHS															
	Warren T. Piver	Chemical Engineer	OHHA NIEHS															
	Herbert S. Posner	Pharmacologist	OHHA NIEHS															
COOPERATING UNITS (if any) Institute-wide																		
LAB/BRANCH None																		
SECTION None																		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, N. C. 27709																		
TOTAL MANYEARS: 0.8	PROFESSIONAL: 0.6	OTHER: 0.2																
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																		
SUMMARY OF WORK (200 words or less - underline)																		
The objectives of this long-term project are: (1) to compile an index of associations between organ system <u>diseases</u> and <u>symptoms</u> in <u>humans</u> and exposure to environmental and occupational chemicals; and (2) to assess the practicality and validity of using <u>neurological</u> and <u>behavioral</u> tests as early indicators of toxicity and as <u>surveillance</u> "tools".																		

PROJECT DESCRIPTION

METHODS EMPLOYED: Preparation of an open-ended data file, which is continually updated via constant literature surveillance along with computer access to various bibliographic and numerical data banks. Membership on committees; attendance at meetings; reviews of documents and manuscripts; evaluation of computerized data files; preparation of reports and monographs; and consultation with scientists from other government agencies, industry, and academia.

MAJOR FINDINGS AND PROPOSED COURSE:

The diseases, syndromes, and symptoms associated with several organ systems have been identified and indexed. A preliminary compilation of associations between exposure to environmental and occupational chemicals and human nervous system diseases, neurological abnormalities, and behavioral symptoms, demonstrate that nervous system dysfunction may result from exposure to a variety of chemical agents. These agents range from gases, heavy metals, chlorinated hydrocarbons, organophosphorus insecticides, and industrial solvents, to a large number of household products. Prenatal and neonatal exposures may have neurobehavioral effects which are not expressed until much later in life. Elderly and "stressed" individuals may also exhibit enhanced susceptibility.

In some instances cause/effect relationships are clearly established, whereas the evidence for other associations is limited to a few anecdotal case reports. Many of the neurological and behavioral symptoms are nonspecific and can be attributed to numerous factors; nevertheless, these symptoms may be the first signs of intoxication with environmental chemicals. The mechanisms of human neurotoxicity remain largely undefined. There is at present a lack of rational criteria for selecting suitable experimental procedures to determine toxic neurobehavioral effects in both animal test systems and human populations. Efforts to compile an index of toxicological symptoms and diseases and to evaluate testing procedures for assessing neurobehavioral toxicity will continue.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A compilation of associations between target organ toxicity and exposures to chemicals will aid the Institute in making a proper assessment of potential health hazards and in identifying the research programs necessary before such assessments can be made. An index of toxicological symptoms and diseases will also assist in the differential diagnosis of diseases considered likely to be of toxicological origin.

PUBLICATIONS

1. Damstra, Terri. Toxicological properties of lead. Environ. Hlth. Perspec. 19: 297-307, 1977.
2. Damstra, Terri. Neurochemistry and Toxicology: Overview. Environ. Hlth. Perspec. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 20007-01 OHHA
PERIOD COVERED October 1, 1977, to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Identification and Evaluation of Environmental Health Hazards: Chemicals and Chemical Carcinogens		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: William Jurgelski, Jr. Medical Officer OHHA NIEHS OTHER: Hans L. Falk Assoc. Dir. for OHHA OHHA NIEHS		
COOPERATING UNITS (if any) Institute-wide		
LAB/BRANCH None		
SECTION None		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, N. C. 27709		
TOTAL MANYEARS: 0.7	PROFESSIONAL: 0.7	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline) The objective of this project is to identify and evaluate real and potential health hazards in the <u>environment</u> with emphasis on <u>chemicals</u> both as <u>toxics</u> and as <u>carcinogens</u> .		

PROJECT DESCRIPTION

METHODS EMPLOYED: Review of existing data in the open literature and in unpublished manuscripts; preparation of reports and monographs; consultation with scientists from other governmental agencies, industry, and academia, both domestic and foreign.

MAJOR FINDINGS AND PROPOSED COURSE: 1. Cancer and Increased Coal Production. An evaluation of the potential impact of increased coal production on cancer incidence in coal miners and of increased coal combustion on cancer incidence in the general population was completed in response to a request from the President's Task Force.

A review of the literature on cancer in coal miners revealed that there was an apparent increased rate of gastric cancer in coal miners over non-miners. Although no definitive conclusion on the association between coal mining and increased risk of cancer of the stomach was possible, the available studies did imply that an increase in coal production would be associated with an increased incidence of stomach cancer in miners. Further work to identify the effects of conflicting variables is required.

In contrast, available epidemiologic studies suggest that the death rate of coal miners from cancer of the lung is appreciably lower than the rate for non-miners of comparable age. These data strongly suggest that an unknown factor in the coal mine environment possibly coal dust exerts a protective effect with regard to cancer of the lung. Accordingly it is anticipated that an increase in the scale of coal mining would not increase the incidence of lung cancer in coal miners. However, if coal dust is in fact beneficial with regard to lung cancer, it is also a causative factor in black lung. Thus while lung cancer rates might not increase as a result of an expansion of coal production, black lung and other respiratory diseases would probably become more prevalent.

A review of the complex and conflicting data on the possible association between air pollution and lung cancer resulted in the conclusion that differences in lung cancer death rates in different populations, i.e. between urban and rural, migrant and home populations, are due to more than cigarette smoking. That is, a so-called "urban" factor associated with air pollution is implicated. The etiologic relationship of air pollution to lung cancer is further suggested by correlations between the concentration of benzo(a)pyrene in air and soil and lung cancer mortality excess in the few studies that have been done in this area. However, an extrapolation based on benzo(a) pyrene levels would appear to be a gross oversimplification considering the importance of adsorptive properties of soots and the synergistic capabilities of air pollutants, particularly those in tobacco smoke.

2. Thresholds in chemical carcinogenesis. A critical review of the carcinogenesis literature has begun with the objective of developing a perspective on

the reality of thresholds in chemical carcinogenesis. The rationale of this undertaking is that adequate hard data is now available to permit a reasoned judgment in this emotionally laden area.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Critical reviews and periodic reevaluations of the type described provide (1) a basis for a more balanced assessment of the risks vs. benefits associated with potential and existing health hazards and (2) reveal those deficiencies and inconsistencies in the available scientific information which require further research and new approaches.

PUBLICATIONS

Falk, H. L. and Jurgelski, W. Jr.: Health effects of coal mining and combustion. Carcinogens and Cofactors. Environ. Hlth. Perspec., 1978 (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 20008-01 OHHA
PERIOD COVERED October 1, 1977, to September 30, 1978		
TITLE OF PROJECT (80 characters or less) The Marsupial Neonate as a Model for the Identification and Evaluation of Environmental Toxicants		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: William Jurgelski, Jr. OTHER: Hans L. Falk L. E. Zimmerman J. M. Henry N. Palmer S. Hoffman	Medical Officer Assoc. Dir. for OHHA Ophthalmic Pathologist Neuropathologist Renal Pathologist Orthopedic Hospital & Seattle, Washington Oral Pathologist	OHHA NIEHS OHHA NIEHS AFIP AFIP The Childrens Med Center Univ. Alabama
COOPERATING UNITS (if any) Medical Arts and Photography, NIH		
LAB/BRANCH Office of Health Hazard Assessment		
SECTION None		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.3	PROFESSIONAL: 0.3	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (a1) MINORS ☐ (a2) INTERVIEWS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER		
SUMMARY OF WORK (200 words or less - underline <		
This project seeks to develop the opossum <u>Didelphis virginiana</u> as a biomedical model by (1) characterizing the normal neonatal and developing opossum anatomically and physiologically and (2) determining the pathophysiologic response of these animals to selected <u>environmental toxins</u> and <u>carcinogens</u> .		

PROJECT DESCRIPTION

METHODS EMPLOYED: Special histologic techniques, light and electron microscopy.

MAJOR FINDINGS AND PROPOSED COURSE:

(1) Opossum Film. Completion of the opossum films (entitled: "The American Opossum as a Biomedical Model". Part I: "The Neonatal and Developing Opossum" and Part II: "The Adult Opossum") continues to be delayed due to unavailability of resources. NIEHS management previously indicated that funds would not be released for the purpose of completing this work until the carcinogenesis work was complete. A request for funds is in preparation.

(2) Carcinogenesis Study. (a) Work continues on the characterization of the lesions induced in developing opossums with ethylnitrosourea (100 mg/kg).

A paper describing the biology and morphology of the spectrum of kidney neoplasms induced is in preparation in collaboration with Dr. N. Palmer of the Childrens Orthopedic Hospital, Seattle, Washington. Travel funds have been obtained from the National Research Council to permit presentation of this work at the XII International Cancer Congress in Buenos Aires, Argentina, in October 1978.

A paper describing the biology and morphology of the oral lesions is in preparation in collaboration with Drs. G. Alderson and S. Hoffman.

A paper describing the biology and morphology of brain tumors is in preparation in collaboration with Dr. J. M. Henry of AFIP. This paper has been accepted for presentation at the VIII International Congress of Neuropathology, Washington, D.C., September 1978.

A paper describing the induced liver lesions is in preparation in collaboration with Dr. J. Hass of the Childrens Orthopedic Hospital, Seattle, Washington. The induced blood dyscrasias are being reviewed by Drs. M. Kaden and S. Petterson, also of the Childrens Orthopedic Hospital, Seattle, Washington.

(b) Collaborative Studies and Consultation. The principal investigator has been asked to collaborate in several studies utilizing the opossum, being conducted or planned at other institutions.

(1) In collaboration with Dr. Bernd Hamprecht of the Max Planck Institute of Biochemistry in Munich, West Germany, a small opossum breeding colony has been successfully established with the objective of reproducing the brain (ganglioglioma) and eye (teratoid medullopithelioma) neoplasms induced with ethyl nitrosourea. (Please see Annual Reports for FY 74 and 75.) Tissue from these two tumor types will be (a) frozen as part of a tumor tissue bank, (b) inoculated into nude mice, and (c) placed in tissue culture for morphological, biochemical and electrophysiological studies of neoplastic neurons in vitro.

(2) The principal investigator functions as a consultant to the Laboratory of Gastrointestinal Physiopathology, Department of Medicine, University of Leuven, (Drs. J. Jaspens and G. Vantrappen), Belgium in a study of the physiology of peristaltic contraction in the esophagus of the opossum.

(3) The principal investigator is a consultant to the Netherlands Central Institute for Brain Research, Amsterdam, The Netherlands, in a comparative morphologic study of the opossum pineal gland (Dr. P. Pevet) and work on nerve regeneration (Dr. R. Baber).

(4) The principal investigator has been asked to collaborate in establishing a research program to reproduce embryonal tumors of the jaw using the opossum neonate by the Department of Pathology, University of Alabama Medical School, Birmingham, Alabama (Dr. S. Hoffman).

(5) The principal investigator has been asked to collaborate in establishing a research project involving the induction of retinoblastic neoplasms in the neonatal opossum at the Eye Institute of Retina Foundation, Harvard Medical School (Dr. Mukai).

(c) Contract Studies. Attempts to re-establish the opossum carcinogenesis study with NCI support continues.

In progress is an attempt by the National Wilm's Tumor Study Group to obtain funds for re-establishing an opossum colony for use in evaluation of therapeutic approaches to Wilm's tumors.

The proposed course of this work is as follows:

(1) Carcinogenesis Study. Histologic preparation of tissues and histopathologic interpretation and classification of lesions will continue as resources allow.

(2) Opossum Films. Completion of the films will depend on the availability of pregnant opossums and technical and fiscal support.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The opossum model provides an opportunity to directly evaluate the relationship between susceptibility to embryonal carcinogenesis and differentiation of the target tissue. The model may also be of value in exploring the apparent inter-relationship among oncogenesis, teratogenesis, and mutagenesis in the absence of the major handicap intrinsic to the eutherian animals; namely, the impossibility of distinguishing direct carcinogen induced teratologic mutagenic changes from identical lesions which are the indirect result of adverse physical and physiologic effects of the carcinogen on the maternal and fetoplacental unit. In the semi-embryonic, semi-fetal opossum orally or parenterally exposed to a carcinogen, mutations and malformations can only be either carcinogen induced or spontaneous.

PUBLICATIONS

Jurgelski, W., Jr., Hudson, P. M., Dunn, R. L., and Falk, H. L.: A new animal model for the direct induction of neoplasms during fetal and embryonic development. Proc. of the 35th International Symposium on Detection and Prevention of Cancer, April 1976. Vol. I, Part I, Ch. 11. H. E. Nieburgs, Marcel Dekker, Inc., New York, N. Y. 1977.

BATTELLE PACIFIC NORTHWEST LABORATORIES - Richland, Washington
(NIH-NIEHS-72-2043)

TITLE: Fate of Heavy Metals and Heavy Metal Complexes in Soils and Plants

CONTRACTOR'S PROJECT DIRECTOR: R. E. Wildung, Ph.D.

PROJECT OFFICER (NIEHS): Warren T. Piver, Ph.D., Chemical Engineer,
Office of Health Hazard Assessment

DATE CONTRACT INITIATED: January 1, 1972

CURRENT ANNUAL LEVEL: \$98,500

PROJECT DESCRIPTION

OBJECTIVES: The objectives of these investigations are to determine using advanced radiotracer, spectroscopic, and chromatographic techniques: (1) the rate and extent of uptake of metals from soils by representative grass and broadleafed plants; (2) the translocation of metals in plants; (3) the effect of metals on soil microbiota and soil microbial processes; (4) the nature of metal bond types and chemical forms of metal metabolites in plant and microbial tissues; and (5) the potential for soil formation of organometal complexes, subsequent uptake of these compounds by plants. Implicit in these objectives are the development of suitable methods for determining the chemical forms of metals in soils, plants, and microbial tissues.

The metals are nickel, cadmium, chromium, and thallium, and the plants are soybeans.

METHODS EMPLOYED: Ritzville soil was thoroughly mixed with labeled and unlabeled isotopes. A split-root (soil/nutrient solution) method was employed to grow soybeans to maturity in the metal containing soils. The plants were cultured in a growth chamber under constant light (14 hr light; 10 hr dark), temperature (27°C light/ 20°C dark) and humidity (40-45%). At maturity the plant tops and roots were harvested (133 and 142 days from planting). Plants were separated by cotyledons, roots, stems, leaves, immature bean pods, mature bean pods and mature beans. In all cases the tissues were analyzed for total metal radioisotopes.

In soil biochemical studies, enrichment techniques have been used to select for microorganisms that exhibit a high resistance to added metal concentrations. Procedures employing TLC, column chromatography, electrophoresis, and mass spectrometry are being developed to identify the metabolites of this enrichment process. These same analytical procedures are being used to characterize the chemical form of the metal in plant xylem, leaves, stems, and fruit.

MAJOR FINDINGS AND PROPOSED COURSE: After screening 17 metals for their uptake by barley (*Hordeum vulgare*) and soybean (*Glycine max*), and their effect of soil microorganisms, intensive studies have focused on nickel (Ni), cadmium (Cd), chromium (Cr), and thallium (Tl). These four metals were chosen because they exhibited the greatest potential for microbial alteration in soil, and uptake and concentration by soybeans.

A. Microbial Studies

Studies with microbial populations have been toward a better understanding of how these organisms transform the four metals added to soil, and the role these processes have in making metals available for uptake by plants. Specific organisms examined in this regard were fungi, actinomycetes, and aerobic and anaerobic bacteria and spore-forming bacteria. Using the four metals, Cd, Cr, Ni, and Tl, an enrichment program was carried out to isolate organisms capable of growing at high metal concentrations. To expand the enrichment studies, a two-phase procedure was developed to select first for organisms on the basis of carbon sources. Then the isolated metabolic types selected on the basis of carbon source were exposed again to Cd, Cr, Ni, and Tl, but over a broader concentration range.

The form of Cd, Cr, Ni, and Tl was characterized in the exocellular fraction of the mixed fungal and bacterial cultures isolated on the basis of carbon requirements and metal resistance. The experiments in this area were further designed to distinguish between those complexation reactions resulting from reaction with metabolites or normal metabolic processes, and those reactions involving the metabolites of metal resistant processes.

As a result of these enrichment studies, it has been demonstrated that growth of organisms with the four metals present, resulted in a change in the chemical form of these metals. These observations have suggested that metabolites formed during growth on different carbon sources, are capable of complexing the metal. These results further suggest that either resistant organisms isolated by these enrichment techniques exhibit an altered metabolism indirectly causing metal modification, or are capable of direct metal modification which may be classified as a detoxification mechanism. Future studies are designed to identify the metal complexes resulting from this transformation. With nickel, the chemical nature of the complexing ligands in soils and plants have been identified.

B. Plant Studies

Chromium was preferentially deposited in the roots, thallium in stem tissue, cadmium in leaf tissue, and nickel initially in the leaves but was remobilized during maturation to seed tissue. 81% of the thallium was associated with the less than 500 molecular weight fraction of stem tissue of soybeans. Whereas 84% of cadmium and 76% of the nickel were associated with molecular weight fractions greater than 10,000 in leaves for Cd and seeds for Ni.

Kinetic studies of nickel uptake have suggested that active accumulation processes using a membrane carrier system employed for nutrient elements is the predominant process for transporting this metal in soybean plants. At

present, investigations are underway to determine if nickel is functioning as a nutrient analog. The results of these studies will shed light on the influence of microbial metabolism on plant availability and ultimate chemical form in the plant. The nickel extracted from the xylem fluid of the kinetic studies had 80% of the total metal associated with molecular weight fractions between 500-10,000. The major portion of this fraction was associated with stable organic compounds consisting of cationic and anionic species of these compounds, several appear to be produced in the root. At present, data are being collected to compare the form of the metal in plants, with the soluble products of microbial metabolism.

C. Biochemical Studies

The actual identification of the chemical nature of these metal complexes extracted from soil fractions, microbial cells, exocellular media and plant components has required the development of new separation procedures. For these reasons, the development of these procedures has been a critical part of this program effort. Techniques and methods of separation which are being applied are ultrafiltration, solvent extraction, electrophoresis, and several types of chromatography. Each method has its application and limitations, and work is continuing to develop methods to provide reproducible results.

PROPOSED Course: The objectives of this continuing program are:

A. Microbial Studies

Using the microorganisms isolated by the enrichment programs, studies will be conducted in three major areas. All of these studies are designed to supply additional information on the mechanisms of modification of metals by soil microbial population. These three areas are:

1. Extension of techniques used to identify aerobic bacteria which show resistance to two or more metals, to identify fungal cultures which exhibit this same capability. Metabolites of the alleged modification of the metals by these particular microbial populations will be identified by techniques outlined in the biochemical studies section.
2. Extension of techniques used to identify aerobic bacteria which show resistance to two or more metals, to identify anaerobic metal-resistant organisms and the mechanism by which this resistance is manifested. Again the role of this process in terms of plant availability will be studied by identification of the products of this modification or resistance-to-the-presence of these metals.
3. Validation of in vitro studies with isolation of resistant micro-organism with soil perfusion column studies. The purpose is to allow evaluation of the importance of the organisms and their metabolites in mediating metal uptake and form in plants.

B. Plant Studies

The three major areas of study are:

1. Analysis of the kinetics of metal uptake, both for each metal itself and for competitive effects.
2. Determination of plant availability of metal complexes produced by soil microorganisms in soil perfusion systems and their potential for translocation to edible components of the plant.
3. Chemical characterization of mobile metal form in xylem exudates and deposited form in tissues of soybean plants following uptake of inorganic and organic metal species.

C. Biochemical Studies

Emphasis will continue on detailed characterization of microbial and plant metabolites. Research will be towards improving the selectivity, sensitivity, and efficiency of methods which have been the most promising. In particular, TLC and TLE have been the most effective in identifying and characterizing metal complexes. Continued research is required to improve solvents, buffers, and chromatographic supports and to examine methodological limitations. It cannot be over-emphasized how important this phase of the work is, and how much it can contribute to the theory of mass separation and characterization of materials by chromatography.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A number of avenues exist by which metals may enter the human food chain. The principal direct avenue probably results from using metals in agriculture as pesticides, desiccants or as contaminants of fertilizers. The importance of soil and plants in this pathway has been underscored by the spectre of metal conversion to more soluble and more toxic heavy metal complexes which may subsequently be taken up by plants and thereby enter directly into human food-stuffs. Particular attention should be given to determining the mechanisms for converting inorganic metals to organic complexes and the extent of this conversion. This information should provide a sound basis for estimating the potential hazard arising from the continued use of these materials and may provide the key to remedial measures.

PUBLICATIONS

Cataldo, D. A., Garland, T. R., and Wildung, R. E.: Nickel in plants.

I. Uptake kinetics using intact soybean seedlings. *Plant Physiology*. (In press).

Cataldo, D. A., Garland, T. R., and Wildung, R. E.: Nickel in plants.

II. Distribution and chemical form in soybean plants. *Plant Physiology*. (In press).

Cataldo, D. A. and Wildung, R. E.: Soil and plant factors influencing the accumulation of heavy metals by plants. *Environ. Hlth. Perspec.* (In press).

MIDWEST RESEARCH INSTITUTE - Kansas City, Missouri 64110
(NIH-NIEHS-72-2090)

TITLE: An Appraisal of Environmental Exposure to Heavy Metals

CONTRACTOR'S PROJECT DIRECTOR: Ivan C. Smith, Ph.D.

PROJECT OFFICER (NIEHS): Warren T. Piver, Ph.D., Chemical Engineer,
Office of Health Hazard Assessment

DATE CONTRACT INITIATED: April 17, 1972

CURRENT ANNUAL LEVEL: \$59,277

PROJECT DESCRIPTION

OBJECTIVES: To identify sources and routes of human exposure to heavy metals as a result of the various steps and industrial operations involved in the commercialization, utilization, and disposal of metals and their compounds. To examine and present available information on the toxicology of the metal and its compounds, and to identify analytical methods and their limitations for quantitative measurement of metals in environmental and biological media.

METHODS EMPLOYED: To perform literature reviews and to seek expert opinions from industry in order to compile data on sources of release and exposure to metals and their compounds. Special interest is placed on determination of the physical-chemical properties of the metal that influence how the metal makes contact with man. The information is put together in a report on each metal of specific interest.

MAJOR FINDINGS AND PROPOSED COURSE: Within the past year, a report on thallium has been produced. These reports have a special significance because they attempt to define the chemical state of the metal in the environment as a result of its release due to intended use or as a contaminant in air and water. The form of the metal and the route of exposure have an important influence on its hazard potential. This type of information distinguishes these reports from the many other reports being produced on metals. Information is being gathered for a number of metals of importance from a toxicological viewpoint. A report on Indium is in press. These reports have been published in book form and are available to a wide readership.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This surveillance is essential to stay abreast of changes in environmental contamination by certain industrial or communal practices involving different metals or different forms of metals, or their complexes. The information will determine whether research on the health effects of the compound in question should be undertaken and in which direction it should go. In addition, these documents

have been sent to biomedical researchers who are with Federal and academic programs, and to program directors in foreign countries. Comments received from these people have indicated that these reports are timely and provide useful information for clinical analysts of patient exposure to metals, and for planning research programs.

PUBLICATIONS

1. Smith, I. C. and Carson, B. L.: Trace Metals in the Environment. Volume 1 Thallium. Ann Arbor Science Publishers, Inc., Ann Arbor, Michigan, 1977.
2. Smith, I. C. and Carson, B. L.: Trace Metals in the Environment. Volume 2 Silver. Ann Arbor Science Publishers, Inc., Ann Arbor, Michigan, 1977.
3. Smith, I. C. and Carson, B. L.: Trace Metals in the Environment. Volume 3 Zirconium. Ann Arbor Science Publishers, Inc., Ann Arbor, Michigan, 1978.
4. Smith, I. C., Carson, B. L., and Ferguson, T. L.: Trace Metals in the Environment. Volume 4 Palladium/Osmium. Ann Arbor Science Publishers, Inc., Ann Arbor, Michigan, 1978.

INTERAGENCY PROGRAMS

OFFICE OF ASSOCIATE DIRECTOR FOR INTERAGENCY PROGRAMS
Summary Statement

The Associate Director for Interagency Programs is responsible for the following program areas:

US-USSR Cooperation

Collaboration between Soviet and American environmental health scientists is carried out under the auspices of two cooperative agreements between the United States and the Soviet Union. Under the *Medical Science and Public Health Cooperative Agreement*, scientists from both countries are conducting joint research on heart disease, cancer, arthritis, influenza and acute respiratory diseases, and health problems associated with environmental pollution. The Director, NIEHS, is U.S. Coordinator for the environmental health activities under the Health Agreement.

1978 was the sixth year of formal collaboration in environmental health research between the US and USSR. The first year was concerned largely with establishing working relationships and agreeing on areas of joint study. Cooperative research efforts were initiated in the second year of the agreement and involved exchange visits between scientists of both sides. The research results developed during the second and third years of collaboration were presented by American and Soviet scientists at a joint symposium in Riga, Latvia, in December 1974. Scientific results from cooperative research during 1975 and 1976 were presented at the second joint symposium, held in Marineland, Florida, in December, 1976. The results of these symposia were published in both countries. During 1977 and 1978, major workshops were held on the topics: "Embryotoxic and Teratogenic Effects of Chemicals" (Leningrad, November, 1977); and "Biological Effects of Metals" (Cincinnati, February, 1978).

Collaborative research efforts are currently divided into four problem areas aimed at (1) developing methods for the quantitative evaluation of the biological effects of environmental chemical agents; (2) predicting the biological effects of environmental chemical agents; (3) studying the long-term biological effects of environmental chemical agents; and (4) studying the long-term effects of physical factors in the environment.

By the end of 1978, over 50 scientific papers will have been published by American and Soviet scientists on the results of environmental health research conducted under this agreement.

The *Agreement on Cooperation in the Field of Environmental Protection* between the US and USSR addresses some of the most important aspects of problems of the environment, including the study of pollution and its effects on life. A number of agencies participate in this agreement, which is under the general direction of the Administrator, EPA. The Director, NIEHS, serves as HEW representative to the agreement and as Co-Chairman of the working group for one of its areas, the Biological and Genetic Effects of Pollutants. Effort in this area has been focused on the mutagenic potential of environmental

contaminants, the toxic effects of heavy metals in the environment, environmental health hazards associated with extraction, processing and utilization of oil from shale, the effects of environmental pollution on the neuro-endocrine system with special emphasis on human behavioral effects, and the use of marine organisms to study the biological and genetic effects of pollutants and as models for biomedical problems.

During 1978, program activity under this agreement consisted of the fourth joint workshop on "Basic and Practical Approaches to Environmental Mutagenesis and Carcinogenesis" held in Baku, USSR, and participation in the joint workshop on the topic "Biological Effects of Metals", held in Cincinnati, Ohio. In addition, exchange visits took place on the topics of health effects of oil shale development, human behavioral effects, and marine organisms.

Energy-Related Research

As part of the high priority accorded by the Federal Government to solving the energy problems facing the nation, NIEHS is involved in a variety of activities to elucidate the potential adverse health impacts of energy technologies.

During the summer and fall of 1974, an OMB/CEQ task force met to identify needed research on the potential adverse health and environmental problems associated with energy use. As part of the report of the Interagency Working Group on Health and Environmental Effects of Energy Use published in November 1974, the Health Sub-Group, co-chaired by the Director, NIEHS, recommended that research be pursued in several areas. As a result of the work of this task force, NIEHS was assigned the responsibility, and given additional funds, to develop research programs to address a number of important energy-related questions. The energy-related research being pursued by NIEHS focuses on mutagenic effects; teratogenic and reproductive effects; behavioral and neurotoxic effects; inhalation toxicity and pulmonary effects; subcellular, cellular and organ toxicity; pharmacologic effects and the determination of immediate and long-term effects of critical pollutants on selected ecosystems and organisms in the marine environment. During the period 1975-1978, NIEHS initiated a number of new projects focused on problems in these areas.

In January 1976, NIEHS co-sponsored, along with NIOSH, EPA, and ERDA, a retreat for 150 scientists working on energy-related health problems. In May 1977, the President directed HEW, EPA, and DOE to establish a joint program to identify the health and environmental effects of energy technology. Working with staff of NIOSH, EPA, and DOE, a Memorandum of Understanding was developed among the agencies concerned and an Interagency Coordinating Committee for Energy-Related Health and Environmental Effects Research was chartered. In addition, the three agencies initiated a series of jointly sponsored scientific workshops for the purpose of obtaining an up-to-date identification of the health and environmental problems associated with each energy technology and the unmet research needs required to address those problems. In April 1977, the President's Message on Energy announced the intention to appoint a special committee to study the health and environmental effects of increased coal production and use. The Director, NIEHS, was appointed

Chairman of the Committee which reported its findings and recommendations to the President in December, 1977.

Interagency Coordination

During 1976-1977, a report on Federal Agency Support for Environmental Health Research was compiled at the request of the Senate Appropriations Committee. This report was updated in June, 1978 at the request of the Director, Office of Science and Technology Policy, Executive Office of the President. The report summarizes the environmental health research responsibilities, functions, and coordination efforts of the Department of Health, Education, and Welfare, Department of Energy, Environmental Protection Agency, Department of Agriculture, Department of Commerce, Department of Interior, Department of Defense, National Science Foundation, Nuclear Regulatory Commission, National Aeronautics and Space Administration, Department of Housing and Urban Development, Department of Transportation, and Veterans Administration. These agencies have budgeted approximately \$587 million for environmental health research in FY 1979.

PROGRAM PLANNING AND EVALUATION

OFFICE OF PROGRAM PLANNING AND EVALUATION
Summary Statement

NIEHS established the Office of Program Planning and Evaluation (OPPE) in 1976, but did not begin staffing it until the Fall of 1977, when the office director returned from a year-long tour as an American Political Science Association Congressional Fellow. Initial staffing consisted of the director, one program analyst, and a secretary. In mid-June 1978, OPPE added a Presidential Management Intern in Public Affairs to its staff. Throughout the year, a consultant, with a background in epidemiology, health care services, and health policy analysis, assisted the office. Plans for early expansion of the staff include an increase in the clerical support staff. Further growth of the professional staff will have to await future fiscal years.

In this first year of operation OPPE has carried out activities in all areas of its responsibility. These have been both self-initiated and externally generated. In addition some have been of a continuing formal nature, such as the forward planning and program evaluation processes, whereas others have been one-time or short-term in nature. The end of this first year of its operation would seem to be an appropriate time for an explication of operational philosophy.

The formal mission of the OPPE is to plan, develop, and direct the Institute program planning, analysis, and evaluation activities. Conceptually, however, the role of the OPPE is to serve as a focus for developing and describing the ways in which the Institute's programs fit into the total array of environmental health activities carried on throughout government. It is only through understanding the universe of environmental health activities that the Institute can effectively develop its own program plans. Thus, to the extent possible, the OPPE attempts to function at the macro level of environment, health, and public policy rather than the micro level of specific research project planning. This approach is in the tradition of the Institute's planning activities.

This is illustrated by NIEHS' support of the First Task Force on Research Planning in Environmental Health Science, a task force established by the Institute's advisory council shortly after NIEHS' creation in 1966, to examine the needs, goals, and resources appropriate to the NIEHS research programs in the years ahead. A large part of this effort involved evaluating existing environmental health research programs, including those of the Institute. The report prepared by the task force ("Man's Health and the Environment--Some Research Needs," published in

1970) assisted Institute staff in selecting particular program areas for special effort and expansion and led to NIEHS' current emphasis on programs in behavioral and neurological toxicology, chronic organ toxicity, reproduction and development, pharmacokinetics, mutagenesis, and toxicity testing.

Program Planning Activities

Recently the Second Task Force on Research Planning in Environmental Health Science completed its work. NIEHS charged this task force with updating the previously mentioned 1970 task force report and with extending its coverage beyond that of the first by including manpower requirements and training. In its report ("Human Health and the Environment--Some Research Needs," published in 1977), the task force identified some major needs in the environmental health research area NIEHS plans to address.

Because of the breadth of the task force's review, the recommendations contained in the report are not limited to the programs of the NIEHS. Rather they range across the entire field of environmental health and by implication the various Federal programs with responsibilities in this area. In order to convert this report to a set of program plans for the Institute, OPPE recommended that a Program Planning Work Group be established to develop specific program proposals with an array of alternatives and funding plans. The NIEHS Director established such a group composed of members of the National Advisory Environmental Health Sciences Council (NAEHSC), the Board of Scientific Counselors, scientists in the Institute's intramural and extramural programs, and staff of the Office of Program Planning and Evaluation.

The Planning Work Group, which is augmented by other Federal and non-Federal scientists, is undertaking an extensive planning and evaluation effort. It is accomplishing this task through three subgroups concerned with: (1) human exposure and its evaluation, (2) injury patterns and mechanisms, and (3) manpower requirements and training. The preliminary report of the group is scheduled for completion by mid-September 1978 and will serve as a framework for developing the 1981-1985 Forward Plan. The final report should prove valuable to the Institute in determining research priorities and program emphases for the coming years. (Primary staff support to this effort is being provided by Extramural Programs.)

Another activity with which OPPE has been concerned is the attempt by various levels of the Federal establishment to cope with the problem of determining how best to coordinate activities in the environmental field. Currently, the Office of the Assistant Secretary for Health, HEW, is examining the creation of a unified environmental health policy for the Department, an essential precondition to offering the State and local counterparts the leadership and technical assistance they need to resolve environmental health problems. As the Federal agency primarily responsible for safeguarding the nation's health through its Public Health Service (of which NIEHS is a part), HEW must be able to provide other agencies effective guidance on the health implications of their policies and decisions.

As part of this activity, OPPE developed a position paper regarding the Role of the Public Health Service in Environmental Health, which was submitted to HEW by the Director, NIH. It stressed that although functions and responsibilities vary widely, the PHS agencies are already carrying out, through their collective efforts, the necessary components of a total environmental health program, namely: research, testing, surveillance, data and information collection, technology transfer, health services, health education, manpower development, and regulation. Moreover, these agencies' programs cover the diverse factors--including chemical and biological substances, infectious agents, injuries, and psycho-social factors--that affect physical, mental, and emotional health which must be encompassed in an overall PHS approach to environmental health.

But to convert this effort into an effective approach to environmental health problems, a central focus is needed for institutionalizing communication among the PHS components, for exchanging information, and for coordinating activities. It was proposed that this function could be carried out effectively through the DHEW Committee to Coordinate Toxicology and Related Programs, for which the NIEHS Director serves as Chairman. It also was proposed that prevention of environmentally-related diseases should be a primary concern of the Public Health Service.

OPPE also has been involved in attempts by the Federal establishment to focus on specific policy areas related to a Federal environmental strategy. The four regulatory agencies in the environmental area (Environmental Protection Agency, Food and Drug Administration, Occupational Safety and Health Administration, and Consumer Product Safety Commission) have formed the "Interagency Regulatory Liaison Group" (IRLG) and entered into a cooperative effort to streamline the regulatory process, to coordinate their efforts, and to improve the protection of worker and public health. They have met with the four research agencies concerned with environmental health problems (National Institutes of Health, National Cancer Institute, National Institute for Occupational Safety and Health, and NIEHS) and plan to develop closer coordination with them. In this regard, OPPE prepared an analysis for the IRLG of the Institute's intramural and extramural research activities that relate to the needs of the regulatory agencies. The IRLG will use this analysis as the basis for its efforts to determine what needs of the regulatory agencies are being met, and where gaps in knowledge exist.

Program Evaluation and Analysis Activities

Evaluation is undertaken to obtain professional and judgemental data on program progress and to periodically reassess programs to determine their potential benefit, cost, impact, and management effectiveness. Achievement of NIEHS's mission and goals is dependent upon the effective evaluation of virtually every facet of its activities.

Because the Institute views the planning and evaluation process as a whole, the development of NIEHS evaluation plans occurs in parallel with the development of the Institute's planning documents, including implementation and forward plans. Evaluations are then conducted in a variety of ways, ranging from project reviews through individual program evaluations to

assessments of the overall Institute program, in support of program management at all levels of the program.

In carrying out its evaluation activities, NIEHS relies to a great extent upon its formally constituted non-governmental review groups, the National Advisory Environmental Health Sciences Council and the Board of Scientific Counselors. In addition, the NIH-wide scientific peer review process serves as an integral part of the Institute's program evaluation system. NIEHS has also developed procedures for vigorous internal review and evaluation of its ongoing research programs by the director, scientific director, associate directors, laboratory and program leaders, and other in-house staff. Moreover, as a useful adjunct device, the Institute encourages individual program leaders to invite non-governmental scientists to evaluate parts of their program.

In the FY 1979 NIEHS Evaluation Plan, OPPE proposed an evaluation of the NIEHS training program in environmental toxicology. The overall purpose of the proposed study is two-fold:

- To evaluate the impact of the existing NIEHS training program and policies in environmental toxicology, and
- To develop baseline data against which to later evaluate the effectiveness of the Institute's training mechanisms, whether extant, new, or improved.

This study is critical due to the increasing importance attached to this area of training, particularly as it relates to implications for implementing the Toxic Substances Control Act; the necessity for verifying the projected manpower requirements in this field; and the need to determine whether manpower trained through the Institute's current programs meet the needs in the environmental toxicology area. From the data obtained in the project, NIEHS wants to acquire a complete analysis of the current environmental toxicologist supply/demand situation, as well as projections of future needs based on estimates received in the survey. It also wants to develop baseline data against which to evaluate the effectiveness of its new "retraining" program to encourage lateral entry of senior research scientists into careers in environmental health sciences as a mechanism for increasing the manpower supply in environmental toxicology.

Another activity, typical of those ad hoc activities carried out by OPPE, concerned the preparation of briefing material for a Government Accounting Office (GAO) study of the facility requirements for the National Center for Toxicological Research (NCTR) and the NIEHS. Specifically, OPPE prepared material on planned changes in research activities and the need for additional facilities for the next 10 years, estimated costs of the planned facilities, and potential for unnecessary overlap of research between the two organizations. The GAO team concluded its study with a report stating that it did not identify any unintentional overlap of research between NIEHS and NCTR although the missions of these two organizations are similar. GAO also reported that some research duplication or overlap is necessary and desirable to verify test results and serve as groundwork for further research.

Legislative Analysis Activities

The NIEHS must maintain continuing awareness of developments in the legislative and executive branches that could impact on its programs, and their relationships to other agencies and organizations. Thus, OPPE (with support from the Division of Legislative Analysis, NIH) operates a legislative information and analysis service, designed to keep the Institute informed of legislative developments in Congress and to ensure NIEHS input into legislative and executive matters of interest and concern to the Institute.

OPPE activities in relation to the legislative information and analysis service are wide-ranging and diverse. They range from developing and maintaining a legislative library dealing with topics of immediate or potential interest to the Institute and key staff (such as health manpower and recombinant DNA research), to preparing memos of alert for NIEHS as well as NIH staff regarding the implications and requirements of proposed legislation. They also encompass tracking an array of bills through the congressional process; researching the background of proposed and enacted legislation; preparing recommendations for NIEHS, NIH, HEW, and Congress on the same; monitoring legislative hearings and developing briefing summaries for staff; editing hearing transcripts and preparing material to be inserted into hearing records; developing statements, briefing books, and background material for testimony by the Institute director, as well as NIH officials and other departmental representatives; and preparing briefing information for congressional staff.

In this regard, OPPE took part in developing the HEW legislative proposals package for the Office of Management and Budget (OMB). The Institute identified the creation of a statutory base for NIEHS as its major proposal for this package. Although the authorities of the NIEHS are similar to those of the other NIH institutes, NIEHS and its mission are not explicitly identified in law. The HEW Secretary created NIEHS by administrative action in 1966 to be the principal Federal agency for research on the effects of chemical, physical, and biological environmental agents on human health and well-being. But NIEHS responsibilities have increased over the years with the enactment of such laws as the Toxic Substances Control Act and the Clean Air Act Amendments. The agencies administering these laws, other Federal agencies, and the Congress frequently raise questions relating to a statutory base for various NIEHS activities; and a specific concern raised is the lack of a well-defined mission for the Institute. It is believed that if NIEHS were provided with a statutory base it would alleviate these concerns and questions.

The statutory authority proposed for NIEHS in the HEW package includes provisions for responsibility for coordination of environmental research activities with other Federal agencies and the facilitation of relevant scientific and technical information exchange on a timely basis; inclusion of representatives from regulatory agencies in the membership of the Institute's advisory council; requirements for a national research plan for environmental health research; authorization for training in environmental health sciences beyond the traditional research training to prepare environmental scientists to meet national needs for testing of chemicals and other environmental agents for health effects; and language

to clarify the role of NIEHS in responding to environmental emergencies, such as the Kepone exposure incident in Hopewell, Virginia.

As another example, OPPE analyzed the Clean Air Act Amendments of 1977 and prepared a memo of alert to the NIEHS director and the NIH Office of Legislative Analysis regarding the NIH responsibilities for the long term. The Clean Air Act Amendments will place a number of demands upon NIH and HEW; and this memo was intended to advise NIH of the need for developing an overall strategy so that effective coordination of NIH's response to the law could occur. Eventually, the memo went to the NIH Policy Development and Implementation Committee which reviewed the Clean Air Act Amendments and attempted to coordinate the activities of the various committees and task forces provided by the law. Among other provisions, this law calls for establishing a coordinating committee (to be chaired by the Environmental Protection Agency and to include representatives from NIEHS, as well as other agencies) to ensure coordination of Federal agencies' research on all substances, practices, processes, and activities which may affect the stratosphere, especially ozone in the stratosphere; and on direct or indirect effects upon the public health and welfare of changes in the stratosphere. The law also requires establishment of a Task Force on Environmental Cancer and Heart and Lung Disease, which includes a representative from NIEHS. One charge to this task force is to recommend a comprehensive research program to determine and quantify the relationship between environmental pollution and human cancer and heart and lung disease, as well as other diseases associated with environmental pollution.

Finally, OPPE has taken part in a number of efforts that are resulting in closer coordination of research and regulatory activities in the environmental health area. Its staff, for example, prepared statements for the NIEHS director on radiation health and safety and on environmental research and development issues for testimony before the Senate and House, respectively. In addition OPPE prepared testimony for the director which supported the proposal in the Drug Regulation Reform Act of 1978 that safety and efficacy data submitted to the FDA be made public.

Related Professional Activities

A number of times during the year the Director, OPPE presented seminars related to Congressional operations. He spoke at Civil Service Commission Seminars, the Research Triangle Federal Executive's Council, and before internal NIH staff groups including the NIH Director's staff.

Publications

During the year, Miss James and Mr. Kingman prepared an article on the development of NIEHS which was accepted for publication in ENVIRONMENTAL HEALTH PERSPECTIVES.

INTRAMURAL RESEARCH

OFFICE OF THE SCIENTIFIC DIRECTOR

OFFICE OF THE SCIENTIFIC DIRECTOR
Summary Statement

In its second full year of operation, there was a change in command early in the fiscal year as Dr. James R. Fouts resigned the position of Scientific Director to return to his previous role as Chief of the Laboratory of Pharmacology. In the interim, Dr. David G. Hoel, Chief of the Biometry Branch, agreed to serve as the Acting Scientific Director. A search committee of both Federal and non-Federal scientists, chaired by Dr. J.E. Rall, Scientific Director, NIAMDD, was formed by the Director and has recommended a list of qualified candidates for the post to the Director. Several of these candidates have or will be interviewed for the position. Dr. Larry G. Hart transferred from the Laboratory of Pharmacology to the Scientific Director's Office to serve as Assistant to the Scientific Director.

Much effort during the year was devoted to (1) recruiting leaders for new or existing programs (Environmental Epidemiology, Pharmacokinetics, and Environmental Mutagenesis); (2) coordinating and reviewing research plans and progress in the basic and applied research programs of the Institute's intramural laboratories and establishing program priorities; (3) working with the Associate Director, Research Resources and program leaders to develop detailed plans for the permanent facility including physical location of programs, special requirements, support services, and animal needs; (4) developing plans for review of programs by the Board of Scientific Counselors; and (5) developing plans for maintaining awareness of contract programs, energy-related research and extramural programs.

Accomplishments include the recruitment of Dr. Stephen Brown to head the Epidemiology Group; the splitting of the large mutagenesis effort into two laboratories (Laboratory of Environmental Mutagenesis and Laboratory of Biochemical Genetics) to allow for more effective management of the program, and formation of a search committee to insure recruitment of a Chief for the Laboratory of Environmental Mutagenesis; and the Pharmacokinetics Section was elevated to Laboratory status and a search committee was formed to seek a Laboratory Chief. Several committees were established, including one to set criteria, review candidates and make recommendations to the Scientific Director on the proposed promotion of nondoctorate level personnel from GS-9 to GS-11; a committee to set criteria and review candidates for conversion from biological laboratory technician to biologist or chemist; an ad hoc committee of scientists representing each laboratory or branch charged with looking into problems and deficiencies within the laboratories, branches and overall intramural program and then making recommendations to the Scientific Director and other program leaders. A chemical selection committee was formed to develop priorities for chemicals to be tested in the Environmental Mutagenesis Test Development Program.

The Board of Scientific Counselors met twice during the year to review intramural programs. Programs presented to and reviewed by the Counselors included the Biometry Branch, the Marine Pharmacology and Biomedicine Section of the Laboratory of Pharmacology, the Chemistry Section of the Environmental Biology and Chemistry Branch, the Laboratory of Pharmacokinetics, and the Environmental Mutagenesis Test Development Program within the Laboratory of Environmental Mutagenesis. The Counselors will be reviewing each of the intramural laboratories and major programs at least once every three years and sometimes more often. The practice of supplementing the Board of Scientific Counselors with ad hoc reviewers in research areas not well represented by Board members will be continued as this results in in-depth reviews of all program areas most efficiently. Further, a formal request has been made to increase the Board membership by two members; i.e., an epidemiologist and a behavioral or neurotoxicologist.

Due to the new research programs begun over the past two years along with expansion or reorganization of existing programs, the Scientific Director's office has spent much time working with the Associate Director for Research Resources and other key persons in reallocating on-site office and laboratory space and seeking off-site space which could be leased and equipped for use by Intramural personnel. The Scientific Director works closely with the Intramural Council and its members (Laboratory and Branch Chiefs in the Intramural and Research Resources programs) to solve various problems that affect the Intramural programs, such as personnel matters and allocation of resources and space.

The Office of the Scientific Director has worked with the Associate Director for Extramural Programs to provide intramural input to the Subcommittee of the NIEHS Advisory Council and the three Program Planning Groups evaluating the recommendations of the Task Force II Report. Intramural scientists are actively participating in each of the Planning Groups which will submit a written report to the Director late in the year.

The Scientific Director continues to meet regularly with other Scientific Directors at NIH in Bethesda to discuss various inter-institute matters such as appointment and promotion plans, policies and reviews, EEO and affirmative action plans, rules for recombinant DNA research, Board of Scientific Counselors reviews of intramural research, travel plans and policies, contracts and monitoring, employment ceilings and grades, central research and research support facilities (libraries, computers, clinical pathology), clinical research activities, post-doctoral and visiting scientist plans, policies and review, personnel management and supervisory training efforts. These meetings are at least twice a month and assure that institutes do not develop procedures and policies at wide variance or threatening to the concept and practices of NIH as a whole. There is also a sharing of information about the research programs at other institutes and this helps in collaboration, avoidance of unnecessary duplication, etc.

The Scientific Director's Office was called upon to present descriptions of the intramural research program to a variety of visitors and to outside groups on a number of occasions, among these were the NIEHS Center Directors, National and international participants at the Comparative Chemical Mutagenesis

Workshop, and a delegation from the North Carolina Student Academy of Sciences. Also, the office coordinated and/or prepared written reports on the Intramural Research Programs in response to requests from various governmental task forces or committees, including the Toxic Substances Strategy Committee and the Task Force on Environmental Cancer and Heart and Lung Disease.

BIOMETRY BRANCH

BIOMETRY BRANCH Summary Statement

The Biometry Branch is primarily involved with applied, environmental-health-oriented research in the areas of biomathematics, risk assessment, and epidemiology. Also, the Branch has responsibility for providing statistical, mathematical, data processing and computer engineering support to the Institute.

Biomathematics research is concerned with the mathematical modelling of biological systems and involves collaboration with a variety of other research groups within the intramural program. Currently, the major areas of particular interest are mathematical population genetics and pharmacokinetic modelling. Risk assessment activities have emphasized improved utilization of data generated from high-dose-level animal screening studies to estimate long-term human risk associated with low environmental levels of exposure. Research is being conducted on a variety of issues such as the evaluation of competing risks and synergistic effects in the framework of multistage models for carcinogenesis, the investigation of species-to-species extrapolation, and the development of mathematical and statistical procedures for low dose extrapolation in toxicology experiments. The Branch's epidemiology program attempts to assess the impact on human health of exposure to potentially hazardous compounds in the environment. Hazard assessments are made directly through actual field studies as well as indirectly by the analysis of pre-established data bases. Methodological research related to the improved planning and evaluation of epidemiological studies is also performed.

Statistical support provided to the intramural program covers a wide range of consulting activities from experimental design and data analysis to model estimation and simulation studies. An active research effort in the development of new analytic techniques is maintained in order to meet some of the highly specialized needs of various Institute scientists. Computer support covers such diverse activities as the creation of information retrieval and inventory maintenance systems, the simulation of complex biological models designed to gain insight into mechanistic processes, and the processing of large volumes of data in conjunction with statistical consulting activities. In addition, computer engineering support is provided for real-time data acquisition and minicomputer controls systems.

BIOMATHEMATICS

At the present time investigations are being conducted in two separate areas of mathematical biology. Each of these programs involves, to some degree, collaboration with other research groups within the intramural program. Joint research with the Laboratory of Environmental Mutagenesis is directed

toward the derivation of more realistic models for certain phenomena in population genetics. As a result of this effort, a new model has been developed for estimating the nucleotide mutation rate through the use of restrictive enzymes. Attempts are being continued to adapt stochastic diffusion equation theory for the prediction of the effect of autocorrelated changes in the environment on gene frequency distributions.

In collaboration with the Institute's program in pharmacokinetics, a large scale project has been initiated to develop kinetic models and methods for quantifying the mechanisms of the uptake, distribution, metabolism, and excretion of xenobiotics in various animal species. Physiological compartmental models of the *in vivo* chemokinetics of different halogenated biphenyls (PCB's) have been constructed for the rat and scaled to the mouse with good results for the more persistent, slowly metabolized PCB isomers. Mathematical models have also been developed to simulate and predict the disposition of these environmental contaminants in various animal species following chronic exposure. In the course of this research a new numerical integration algorithm was perfected. This proved to be much more efficient than previously employed methods for solving multiple-dose pharmacokinetic model equations.

RISK ASSESSMENT

Risk assessment research focuses on the dual objectives of extrapolating the results of high-dose-level animal toxicology experiments to the low levels that typically exist in the human environment, and of estimating the corresponding human health risk associated with long-term exposure at these environmental levels. A variety of related projects have been initiated in the pursuit of these objectives.

The multistage theory of carcinogenesis has been used to derive a number of mathematical models for reflecting the independent and dependent effects of two agents or risk factors acting in combination. When the results obtained from these models were compared to traditional indices for quantifying synergism, the indices were found to be seriously in error in many instances. As a result improved procedures for designing and analyzing multifactor carcinogenicity experiments have been proposed.

Relative human (compared to various animal species) risks associated with exposure to different carcinogens which had been evaluated in the scientific literature were re-examined. It was found that consideration of species lifetimes and body surface areas caused the human and animal risk estimates to be in closer agreement on the average than had been previously observed. A review of other known human carcinogens is being conducted to determine if this parallelism with various animal models is maintained.

The methodology for low dose extrapolation in carcinogenic risk assessment is being modified to deal with experimental endpoints other than carcinogenesis. Initially, attention is being given to toxicological experiments resulting in litter data, such as that encountered in teratology or dominant lethal research. The asymptotic properties of various parametric and non-parametric procedures for detecting the presence of litter effects have been compared, and attempts are being made to extend generalized dose-response

curves to toxicological experiments involving litter effects.

EPIDEMIOLOGY

Direct (field) studies for the identification of potential health hazards in the general environment continue to play an important role in the Biometry Branch's epidemiology program. A prospective study for the evaluation of the possible excess risk incurred by breast fed infants as a result of their exposure to elevated levels of polychlorinated biphenyls (PCB's) in mother's milk has been initiated. Mothers are currently enrolled in participating hospitals, and plans are now being formulated for the long-term follow-up of their newborn children beyond an initial early developmental six-month study period.

A field-based study of the relationship between spontaneous abortion and the exposure of pregnant women to any of a number of defined environmental mutagens and clastogens is now in the planning stage. This particular "disease" endpoint or pregnancy outcome is not only of interest in its own right, but it may also serve as an indicator of other more serious and longer range damage to the human genome, including neoplasia.

A limited record study of the potential effects of lead exposure on the cardiovascular system in children who had measureable body burdens of lead, but who were not clinically intoxicated, has been completed. In contrast to a similar study conducted in an adult population, no positive associations were detected.

Finally, the applied research effort directed toward statistical methodological problems in the field of epidemiology has been continued. Interest has centered on the bias introduced by the use of screening test results to estimate disease prevalence, the potential effects of misclassification on follow-up studies, and on the assessment of synergism associated with the joint action of two separate risk factors.

STATISTICAL CONSULTING

One of the primary functions of the Biometry Branch is to provide a comprehensive statistical consulting service for the intramural research program. This effort covers a wide range of activities in the areas of experimental design and data analysis.

The Branch continues to stress the need for its involvement in the initial phases of laboratory investigations. Consideration is given to factors such as study feasibility, sample size determination, and optimum animal allocation. While the majority of statistical support involves data analysis, collaboration with respect to experimental design is improving.

Data analysis efforts include parameter estimation, curve fitting, and significance testing using a wide variety of parametric and nonparametric procedures. For large data sets or studies requiring the use of complex statistical procedures, data analysis activities are closely coordinated with the Branch's computing work group.

Applied statistical research problems are frequently encountered as a direct consequence of the Branch's consulting activities. For example, the investigation of the stochastic variability in serial dilutions and other experimental procedures employed in mutagenic testing led to the development of new procedures for analyzing microbial data. Other consulting-related methodological research has generated improved nonparametric procedures for ED50 estimation, mathematical modelling for certain binary response variables, new statistical techniques for recessive lethal detection, and an examination of the operating characteristics of several alternative hypothesis-testing procedures in carcinogenicity screening programs.

COMPUTING

The Biometry Branch has the responsibility of providing computing and data processing support to NIEHS. The activities accomplishing this support may be thought of as consisting of two cooperating and interlocking efforts, namely computer operations and programming, and computer engineering.

The computer operating and programming effort assumes the responsibility for maintaining NIEHS' PDP 11/40 computer system and a network of terminals connected to the various computers at NIH/DCRT, assisting the NIEHS community in its use of available computer systems, providing programming consultation services as required, providing software systems development capabilities to support intramural research efforts, and providing support and collaborative assistance to the computer engineering effort.

Scientific programming activities conducted by computing personnel have included development of a laboratory animal data-base system for storing and retrieving a broad range of vital information on an animal-by-animal or experiment-wide basis, including physical parameters, experimental parameters, and a geneologic history of the population. In support of intramural research, assistance has been provided to the Laboratory of Environmental Mutagenesis with methodology development for assessment of mutational damage in the mouse. The Pharmacokinetics section continues its use of computing facilities for analysis of pharmacokinetic data and development of techniques for inter-species extrapolation of low-level, long-term pharmacokinetic profiles. In addition to the above efforts are a multitude of small, short-term tasks performed in support of intramural researchers in nearly every working group in the Institute. Within the Biometry Branch, substantial efforts have been directed toward improving the general data processing capability, particularly as it affects the computing needs of those providing statistical data analysis in support of the intramural research program. The PDP 11/40 computer has been significantly expanded and systems analysis studies have been performed preparatory to its being upgraded by the addition of a PDP 11/70 CPU. This computer is used for high-speed telecommunications with the NIH IBM/370 system, and a great variety of local computing activities.

Development of the capability for providing computer engineering support to the laboratories of the Institute is also ongoing within the Biometry Branch. Solutions are being sought to engineering problems related to all aspects of computer hardware and software. Tasks within this effort have included the specification of minicomputers, peripherals, and vendor-supplied software;

the design of timing devices and interfaces between minicomputers and laboratory instruments; and the development of software for control of experiments, data acquisition, and data transfer. The following efforts are underway or were completed in the last year: (1) design support of computer interfaces; (2) design of a special purpose controller/timer for behavioral studies; (3) computer system support of the development of a scanning microscope for biochemical genetics studies; (4) development of a multi-purpose lab minicomputer adaptable to population genetics studies; (5) development of a minicomputer system for the acquisition of scintillation counter data in support of studies on prostaglandins and non-respiratory lung functions; (6) computer engineering support for the warehouse cash register data link; (7) computer hardware support of the growth of the Biometry Branch central computer (PDP 11/40), including the addition of a dual flexible disk for lab computer compatibility, a 16-line serial multiplexer for adding terminals, and dial-up terminal access for remote support. In addition a variety of computer support for the Behavioral Toxicology computer network has been provided. This broad-ranging support has included the specification of a central data collection machine, and the design of interfaces for the SKED computer systems and the activity-monitoring computer system. Software support has included the development of a variety of programs for manual data entry, experiment control, data collection, data analysis on lab computers, and for interfacing to a data base to be maintained on the Biometry PDP 11/40 computer.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 40002-08 BB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Statistical Methodology Development		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Other:	J. K. Haseman B. H. Margolin B. C. Gladen M. A. Hamilton	Mathematical Statistician Mathematical Statistician Staff Fellow IPA
		BB NIEHS BB NIEHS BB NIEHS BB NIEHS
COOPERATING UNITS (if any)		
LAB/BRANCH Biometry Branch		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0.0
CHECK APPROPRIATE BOX(ES)		
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER		
☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
The purpose of this project is to conduct research on <u>statistical methodology</u> problems related to the Branch's consulting activities. Specific areas in which research is being conducted include statistical aspects of life-time carcinogenicity studies, <u>mathematical modeling</u> of intra-litter association in toxicological experiments, <u>nonparametric multiple comparison procedures</u>, and detection of recessive lethals in mutagenicity studies.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Statistical techniques ranging from Monte-Carlo simulation procedures to analytical test development and mathematical modeling have been employed to address various statistical methodology problems arising from the Branch's intramural consulting activities.

MAJOR FINDINGS AND PROPOSED COURSE: (1) A correlated binomial model was derived for use in certain toxicological experiments with binary response data. The properties of this two-parameter model were compared with the alternative beta-binomial and also with the simpler one-parameter Poisson and binomial models. (2) Monte-Carlo studies suggested that, among several alternative nonparametric multiple comparison procedures, the nonparametric analog to Fisher's LSD (a Kruskal-Wallis test, followed by pairwise Mann-Whitney U tests if a significant overall effect is detected) may be preferable to the Nemenyi-Dunn and Steel-Dwass procedures because of the extreme conservatism of these latter methods. (3) A procedure based on the likelihood ratio test statistic was derived for the detection of recessive lethals in mutagenicity studies. This technique, which has the advantage of taking the pattern as well as the total number of fetal deaths into account, was shown to have greater power than procedures currently in use. (4) In response to recent criticism concerning the use of hypothesis testing in carcinogenicity screening programs, the operating characteristics of several proposed test procedures were investigated. It was found that although the overall type I error may be inflated if multiple-comparison considerations are ignored, certain of the more realistic procedures performed satisfactorily under several different experimental conditions. (5) The design of simulation and distribution sampling experiments was investigated. A strategy for assigning pseudorandom numbers to experimental points consistent with experimental design blocking concepts was devised and shown to improve upon existing recommendations for a wide class of problems. (6) A Monte-Carlo study was conducted to determine the accuracy and precision of the estimated variance of the trimmed Spearman-Kärber estimate of the ED50. The level and accuracy of the associated 95% confidence interval estimates were also calculated and compared to the usual maximum likelihood and Spearman-Kärber estimates for a variety of different experimental conditions.

Statistical methodological research will continue to form an important part of the overall support effort provided to the intramural research program.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

If the quality of statistical consulting provided to the intramural program is to be maintained at a high level, then the development of improved statistical methodology is essential.

PUBLICATIONS

Haseman, J.K.: Exact sample sizes for use with the Fisher-Irwin test for 2x2 tables. Biometrics 34: 106-109, 1978.

Haseman, J.K.: Response to "The use of statistics when examining life-time studies in rodents to detect carcinogenicity" by D.S. Salisburg. J. Toxicol. Environ. Health 3: 633-636, 1977.

Kupper, L.L. and Haseman, J.K.: On the use of a correlated binomial model for the analysis of certain toxicological experiments. Biometrics 34: 69-76, 1978.

Lin, F.A. and Haseman, J.K.: An evaluation of some nonparametric multiple comparison procedures by Monte-Carlo methods. Communications in Statistics, Part B; Simulation and Computation, in press.

Schruben, L.W. and Margolin, B.H.: Pseudorandom number assignment in statistically designed simulation and distribution sampling experiments. Journal of the American Statistical Association, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 40004-01 BB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Statistical Methods in Epidemiology		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Other:	B. C. Gladen M. A. Hamilton M. D. Hogan W. J. Rogan	Staff Fellow IPA Mathematical Statistician Staff Associate BB NIEHS BB NIEHS BB NIEHS BB NIEHS
COOPERATING UNITS (if any) Department of Biostatistics, University of North Carolina		
LAB/BRANCH Biometry Branch		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this project is to conduct research on <u>statistical methodology</u> problems related to the Branch's activities in the field of <u>epidemiology</u> . The objectives are both to broaden understanding of the uses and limitations of currently employed study designs and corresponding analyses; and to develop new techniques for statistical analyses of epidemiological studies.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Statistical techniques for the analysis of various kinds of epidemiological studies were devised or evaluated. Both theoretical mathematical calculations and computer simulations were used to assess techniques.

MAJOR FINDINGS AND PROPOSED COURSE: (1) The common use of positive test prevalence as an estimate of disease prevalence in a screening situation was shown to produce serious overestimates in examples taken from the literature. If independent estimates of sensitivity and specificity are available, a corrected estimate can be obtained, and the properties of such corrected estimates were examined. (2) The potential effects of misclassification on follow-up studies were investigated, with particular reference to the long-term study of the effects of polybrominated biphenyls (PBB's) on human health in Michigan (Contract Y01-CP-60215). Preliminary studies show that the proposed measure of exposure (serum levels of PBB's) will misclassify some subjects. The power of statistical tests to detect relationships between exposure and disease was shown to be relatively unaffected by this misclassification, but estimates of relative risk of disease (particularly highly elevated estimates) were shown to be biased. (3) Comparisons of fourteen 2x2 table measures of association and of six 2x2x2 table measures of synergism were made. The comparisons were based on a conceptual population model for the true association between the disease and the risk factor(s). Each measure was judged as to potential use and identified with the study designs under which it can be estimated. It was shown that prior information about the correct population model is required to adequately measure synergism. (4) A new technique was devised for the detection of synergism in cohort studies, if synergism is defined as interaction on a probability scale. This new test was shown to be more useful in small samples than other proposed alternatives; it was both more accurate and more readily calculable.

The proposed course is to continue research efforts on statistical methodology problems that arise in connection with the epidemiological investigations carried out by the Biometry Branch.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The understanding and criticism of current statistical techniques and the development of improved techniques is important for the proper evaluation of the results of epidemiological studies.

PUBLICATIONS

Rogan, W.J. and Gladen, B.: Estimating prevalence from the results of a screening test. American Journal of Epidemiology. 107: 71-76, 1978.

Hogan, M.D., Kupper, L.L., Most, B.M., and Haseman, J.K.: Alternatives to Rothman's test for synergism (or antagonism) in cohort studies. American

Journal of Epidemiology, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 40005-01 BB								
PERIOD COVERED October 1, 1977 to September 30, 1978										
TITLE OF PROJECT (80 characters or less) Statistical Analysis of Mutagenesis Testing Data										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI:</td> <td style="width: 33%;">B. H. Margolin</td> <td style="width: 33%;">Mathematical Statistician</td> <td style="width: 33%;">BB NIEHS</td> </tr> <tr> <td>Other:</td> <td>E. Zeiger</td> <td>Section Head</td> <td>LEM NIEHS</td> </tr> </table>			PI:	B. H. Margolin	Mathematical Statistician	BB NIEHS	Other:	E. Zeiger	Section Head	LEM NIEHS
PI:	B. H. Margolin	Mathematical Statistician	BB NIEHS							
Other:	E. Zeiger	Section Head	LEM NIEHS							
COOPERATING UNITS (if any) Laboratory of Environmental Mutagenesis										
LAB/BRANCH Biometry Branch										
SECTION										
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709										
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0.0								
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS										
SUMMARY OF WORK (200 words or less - underline keywords) The primary objective of this project is to develop appropriate statistical techniques for the analysis of data arising from the Mutagenesis Testing Program. Initial focus has been on data from microbial test systems. Statistical procedures currently employed by other researchers in mutagenesis for the analysis of microbial data have been studied, and new and improved procedures have been devised.										

PROJECT DESCRIPTION

METHODS EMPLOYED: A large microbial experiment conducted by LEM served as the empirical base for this modeling effort. Existing models, such as the currently favored Poisson model, were analyzed to see if they were consistent with the laboratory observations obtained by LEM. Considerations of stochastic variability in serial dilutions and in other experimental procedures led to new statistical techniques for the analysis of experiments of this type.

MAJOR FINDINGS AND PROPOSED COURSE: (1) The Poisson model frequently adopted for the modeling of certain microbial test data is contradicted by empirical evidence; it seriously underestimates the variability of the counts of revertants observed in the Ames test. (2) A logarithmic transformation of the counts serves to stabilize variability and to permit employment of standard statistical procedures for the analysis of the transformed data. (3) Further validation of the findings in (1) and (2) with data sets from other laboratories is needed. EPA data are in hand, with other data sets from NCI and elsewhere anticipated. (4) Additional empirical and analytical investigations of various compound-Poisson models, especially the negative binomial model, are under way. These models are able to accommodate the hyper-Poisson variability observed and permit more realistic prediction and testing of statistical hypotheses.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This project potentially will alter the ways in which certain microbial test data are analyzed. The new methods of analysis will tend to reduce the percentage of false declarations of positive or negative findings resulting from use of these microbial tests. It is also anticipated that this research effort will yield methodological results of interest to biometrists in numerous other areas of research.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 41001-04 BB																
PERIOD COVERED October 1, 1977 to September 30, 1978																		
TITLE OF PROJECT (80 characters or less) Risk Assessment for Environmental Carcinogens																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																		
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">D. G. Hoel</td> <td style="width: 35%;">Chief</td> <td style="width: 15%;">BB NIEHS</td> </tr> <tr> <td>Other:</td> <td>A. P. Davis</td> <td>Visiting Associate</td> <td>BB NIEHS</td> </tr> <tr> <td></td> <td>M. A. Hamilton</td> <td>IPA</td> <td>BB NIEHS</td> </tr> <tr> <td></td> <td>M. D. Hogan</td> <td>Mathematical Statistician</td> <td>BB NIEHS</td> </tr> </table>			PI:	D. G. Hoel	Chief	BB NIEHS	Other:	A. P. Davis	Visiting Associate	BB NIEHS		M. A. Hamilton	IPA	BB NIEHS		M. D. Hogan	Mathematical Statistician	BB NIEHS
PI:	D. G. Hoel	Chief	BB NIEHS															
Other:	A. P. Davis	Visiting Associate	BB NIEHS															
	M. A. Hamilton	IPA	BB NIEHS															
	M. D. Hogan	Mathematical Statistician	BB NIEHS															
COOPERATING UNITS (if any) Louisiana Tech University, Department of Mathematics																		
LAB/BRANCH Biometry Branch																		
SECTION																		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																		
TOTAL MANYEARS: 1.3	PROFESSIONAL: 1.3	OTHER: 0.0																
CHECK APPROPRIATE BOX(ES)																		
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER																		
☐ (a1) MINORS ☐ (a2) INTERVIEWS																		
SUMMARY OF WORK (200 words or less - underline keywords)																		
The overall purpose of this project is to develop improved statistical techniques for using data from animal carcinogenicity tests conducted at high dose levels to estimate long-term human risk from chemical carcinogens at very low dose levels. Current research efforts have focused on such topics as the evaluation of competing risks and synergistic effects in the framework of multistage models for <u>carcinogenesis</u> as well as the question of <u>species-to-species extrapolation</u>.																		

PROJECT DESCRIPTION

METHODS EMPLOYED: (1) The multistage theory for cancer initiation was used to derive mathematical models for independent and dependent effects of two agents. Then the standard statistical measures of synergism were evaluated in terms of these multistage models. (2) Meselson's NAS estimates of human relative cancer risk--where relativity is expressed in terms of the most sensitive animal species tested--for a variety of known carcinogens were re-examined to determine the extent to which they depend on both the length of exposure and the unit of dosage quantification.

MAJOR FINDINGS AND PROPOSED COURSE: (1) The research into the assessment of the synergistic potential of two carcinogenic risk factors demonstrated that erroneous conclusions concerning effects on the mechanism of the carcinogenic process can be made if the traditional statistical measures are employed. Improved techniques for the design and analysis of multifactor carcinogenicity experiments were formulated as a result of this research. (2) It was found that when relative lifespan and body surface area were introduced into the animal versus man risk calculations for the carcinogens considered by Meselson, the similarity between species was even stronger than he originally indicated. Other known human carcinogens are now being evaluated to determine if this trend persists. (3) Research into the issue of competing risks is also being initiated. This research departs from the usual competing risk framework by assuming that for each animal (human) under study only the probability that death can be attributed to the specific cause of interest (as opposed to a confirmed cause of death statement) is known.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

As animal experimentation plays an ever-increasing role in the assessment of human cancer risk, the importance of improved statistical procedures for realistically assessing this risk is obvious.

PUBLICATIONS

Guess, H.A., Crump, K.S., and Peto, R.: Uncertainty estimates for low dose rate extrapolations of animal carcinogenicity data. Cancer Res. 37: 3475-3483, 1977.

Guess, H.A. and Hoel, D.G.: The effect of dose on cancer latency period. J. of Environ. Path. and Tox. 1: 279-286, 1977.

Crump, K.S., Guess, H.A., and Deal, K.L.: Confidence intervals and test of hypotheses concerning dose response relations inferred from animal carcinogenicity data. Biometrics 3: 437-449, 1977.

Guess, H.A. and Crump, K.S.: Best estimate low dose extrapolation of carcinogenicity data. Environ. Health Persp. 22: 149, 1978.

Guess, H.A. and Crump, K.S.: Maximum likelihood estimation of

dose-response functions subject to absolutely monotonic constraints.
The Annals of Statistics 6: 101-111, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 41003-01 BB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Low Dose Extrapolation in Noncarcinogenic Toxicological Experiments		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: P. I. Feder IPA BB NIEHS		
COOPERATING UNITS (if any)		
LAB/BRANCH Biometry Branch		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The focus of this research effort is on the generation of mathematical and statistical procedures applicable to the problem of <u>low dose extrapolation</u> in toxicological experiments involving endpoints other than carcinogenicity. Emphasis is given to the development of optimal procedures for ascertaining the presence of <u>litter effects</u> in laboratory data and to the extension of the Prentice generalized <u>dose-response curves</u> to toxicological experiments involving litter effects. The results of extrapolation based on biological theory are to be compared to those based on empirical models.		

PROJECT DESCRIPTION

METHODS EMPLOYED: The methodology for low dose extrapolation in carcinogenic risk assessment is being modified to deal with noncarcinogenic phenomena and to take into account potential correlations among responses within litters for toxicological experiments resulting in litter data. In addition, various statistical techniques including mathematical analysis and numerical evaluation of distributional properties have been utilized to compare alternative statistical procedures for assessing the presence of litter effects in laboratory data.

MAJOR FINDINGS AND PROPOSED COURSE: (1) The asymptotic properties of a procedure for detecting litter effects that is based on the beta binomial model (which allows the investigator to quantify the extent of litter effects) was compared to nonparametric procedures based on the Chi square tests for homogeneity and goodness-of-fit, respectively. It was found that the beta binomial test performed nearly as well as the better of the two nonparametric techniques, i.e., the Chi-square test of homogeneity, even when the assumption of an underlying beta binomial distribution was violated. (2) Current research is centered on attempts to demonstrate that different dose-response curves may fit high dose noncarcinogenic laboratory data about equally well yet result in extrapolated low dose risks differing by orders of magnitude. One approach to the resolution of this type of anticipated discrepancy may be the utilization of generalized dose-response curves such as those described by Prentice. (3) Extensions of this research will involve the derivation and fitting of dose-response functions based on simple mechanistic models. The results of extrapolations based on biological theory will be compared with those derived from empirical models.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This work will advance the techniques of toxicological risk assessment to a level more comparable to that currently attained for carcinogenesis problems. Practical aspects of this research could influence the regulatory approach toward safety evaluation of potentially toxic substances.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 42002-06 BB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Pharmacokinetic Modeling and Methodology Development		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Other:	D. B. Tuey H. B. Matthews N. M. Morales R. L. Dedrick R. J. Lutz	Staff Fellow Research Chemist Visiting Fellow Section Head Chemical Engineer BB NIEHS LPK NIEHS LPK NIEHS BEIB DRS BEIB DRS
COOPERATING UNITS (if any) Chemical Engineering Section, BEIB, DRS, NIH Laboratory of Pharmacokinetics		
LAB/BRANCH Biometry Branch		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.8	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this project is to develop <u>pharmacokinetic models</u> and <u>methods</u> for quantitating the <u>mechanisms</u> of the <u>uptake</u> , <u>distribution</u> , <u>metabolism</u> , and <u>excretion kinetics</u> of <u>environmental xenobiotics</u> in various animal species. <u>Mathematical models</u> of the <u>in vivo pharmacokinetics</u> of several <u>halogenated biphenyls</u> in the rat have been developed and have been scaled to the mouse. Other models have been constructed to describe the disposition of these environmental contaminants in animals following chronic exposure. The pharmacokinetics of xenobiotics in marine animals that serve as human food sources is also being modeled. These mathematical models are intended to provide functional strategies for helping to extrapolate the disposition of chemical contaminants in the environment from one compound to another, and from one animal species to another, ultimately to include man. The evaluation and implementation of existing techniques and the development of new methodologies needed to accomplish these objectives are an integral part of this project.		

POSITION DESCRIPTION

METHODS EMPLOYED: The physiologic modeling approach used in drug disposition is being employed to develop similar type models for environmental contaminants. Computational algorithms for solving the equations that describe these models are being implemented.

MAJOR FINDINGS AND PROPOSED COURSE: (1) Physiological compartmental models have been constructed to describe the individual disposition kinetics of several different polychlorinated biphenyl (PCB) compounds in the rat. The tissue level time courses predicted by the models were generally consistent with the experimental data. Intestinal reabsorption was significant for metabolites of the lower chlorinated compounds. Animal growth was found to have a significant dilution and re-distribution effect on the tissue levels of the slowly metabolized, highly lipophilic 2,4,5,2',4',5'-hexachlorobiphenyl compound. Skin tissue may not be perfusion-limited as assumed in the present model. Metabolism is the rate-determining step for the elimination of these compounds. The pharmacokinetics of 3,5,3',5'-tetrachlorobiphenyl showed that the positions of biphenyl chlorination can have more effect on the rate of metabolism than the degree of chlorination. (2) When allometric equations were used to scale these models to predict disposition kinetics in the mouse; good results were obtained for the more persistent, slowly metabolized isomers. (3) Whole body elimination kinetics after a single i.v. dose to mice was used to predict the body burden in other mice that received multiple oral doses of the same PCB compound. (4) A physiological compartmental model constructed from data obtained from rats that received a single i.v. dose of 2,4,5,2',4',5'-hexabromobiphenyl was able to simulate and predict the tissue levels found in rats placed on a 30-day chronic oral dose schedule. (5) It appears feasible that a perfusion-limited physiological pharmacokinetic model can describe the disposition kinetics of octane and hexadecane in the lobster, which possesses a semi-open circulatory system. (6) A variable order, variable step-size numerical integration algorithm was found to be both computationally stable and several orders of magnitude more efficient than previously used predictor-modifier-corrector methods for solving multiple-dose pharmacokinetic model equations.

Future work will include attempts to refine the PCB models and account for small discrepancies which may prove significant in long-term studies. Compound-to-compound extrapolation of long-term PCB disposition kinetics based on short-term excretion kinetics and chlorination pattern will also be investigated. Mathematical models will be developed for the pharmacokinetics of octane and hexadecane in the lobster, and a general purpose numerical integration algorithm will be implemented on this Institute's computer system.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

An ability to quantitate pharmacokinetic similarities and differences from species to species and from compound to compound will enable better judgements to be made of the relevance of animal study results to man.

PUBLICATIONS

Lutz, R.J., Dedrick, R.L., Matthews, H.B., Eling, T.E., and Anderson, M.W.: A preliminary pharmacokinetic model for several isomers of polychlorinated biphenyls in the rat. Drug Metab. Dispos. 5: 386-396, 1977.

Tuey, D.B. and Matthews, H.B.: Pharmacokinetics of 3,3'5,5'-tetrachlorobiphenyl in the male rat. Drug Metab. Dispos. 5: 444-450, 1977.

Matthews, H.B., Kato, S., Morales, N.M., and Tuey, D.B.: The distribution and excretion of 2,4,5,2',4',5'-hexabromobiphenyl, the major component of Firemaster BP-6^R. Toxicol. Environ. Health 3: 599-605, 1977.

Anderson, M.W., Eling, T.E., Lutz, R.T., Dedrick, R.L., and Matthews, H.B.: The construction of a pharmacokinetic model for the disposition of polychlorinated biphenyls in the rat. Clin. Pharmacol. Therap 22: 765-773, 1977.

Matthews, H.B., Tuey, D.B., and Anderson, M.W.: Pharmacokinetic models for lipophilic compounds. Environ. Health Perspect. 20: 257-262, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 42003-01 BB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Pharmacodynamic Dose-Response Relationships		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	D. B. Tuey D. G. Hoel	Senior Staff Fellow Chief
		BB NIEHS BB NIEHS
COOPERATING UNITS (if any)		
LAB/BRANCH Biometry Branch		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0.0
CHECK APPROPRIATE BOX(ES)		
☐ (a) HUMAN SUBJECTS		
☐ (b) HUMAN TISSUES		
☒ (c) NEITHER		
☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
The purpose of this project is to determine whether pharmacodynamic considerations can provide insights for improving the <u>extrapolation</u> of experimental dose-response data: (1) from high- to low-dose; (2) from animals to man; and (3) from one compound to another. Pharmacokinetic and biochemical mechanism models are examined to evaluate their effects on statistical risk assessment models.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Pharmacokinetic models of xenobiotic disposition in the body, and various biochemical mechanisms of metabolic activation and deactivation are examined in the context of their real or potential ability to alter probabilistic dose-response models derived from statistical methodology.

MAJOR FINDINGS AND PROPOSED COURSE: (1) Pharmacokinetic dose-response effects are highly dependent upon the relative metabolic activation and deactivation rates of the underlying biochemical processes. (2) Biological steady-state levels are not identical to equilibrium results. For linear pharmacokinetic models the existence of a linear relationship between the external and internal steady-state dose rate depends on the connectivity of the system.

The proposed course is to continue to study the pharmacodynamics of dose-response relationships, particularly those which are associated with saturable mechanisms of metabolic activation and deactivations.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This project will provide better understanding of the pharmacodynamic bases of dose-response curves needed to assess the validity of estimating low-dose human risk from high-dose animal experiments.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 43001-06 BB																
PERIOD COVERED October 1, 1977 to September 30, 1978																		
TITLE OF PROJECT (80 characters or less) Demographic Investigations of Potential Human Health Hazards																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">M. D. Hogan</td> <td style="width: 35%;">Mathematical Statistician</td> <td style="width: 15%;">BB NIEHS</td> </tr> <tr> <td>Other:</td> <td>P. Y. Chi</td> <td>Visiting Fellow</td> <td>BB NIEHS</td> </tr> <tr> <td></td> <td>L. C. Clark</td> <td>IPA</td> <td>BB NIEHS</td> </tr> <tr> <td></td> <td>D. G. Hoel</td> <td>Chief</td> <td>BB NIEHS</td> </tr> </table>			PI:	M. D. Hogan	Mathematical Statistician	BB NIEHS	Other:	P. Y. Chi	Visiting Fellow	BB NIEHS		L. C. Clark	IPA	BB NIEHS		D. G. Hoel	Chief	BB NIEHS
PI:	M. D. Hogan	Mathematical Statistician	BB NIEHS															
Other:	P. Y. Chi	Visiting Fellow	BB NIEHS															
	L. C. Clark	IPA	BB NIEHS															
	D. G. Hoel	Chief	BB NIEHS															
COOPERATING UNITS (if any) 																		
LAB/BRANCH Biometry Branch																		
SECTION 																		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																		
TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.6	OTHER: 0.0																
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINDS ☐ (a2) INTERVIEWS																		
SUMMARY OF WORK (200 words or less - underline keywords) The overall objective of this project is to identify and/or confirm the presence of various potential health hazards in the general environment through the mechanism of demographic investigations. Current research activities are concerned with the development of <u>computerized mapping techniques</u> for assessing <u>geographic variability</u> in various indices related to <u>pregnancy outcome</u>.																		

PROJECT DESCRIPTION

METHODS EMPLOYED: Computer routines are being developed to generate maps of various indices of pregnancy outcome calculated on a per county basis (using the state of North Carolina as a model). Algorithms to compare individual county indices with overall, average rates (assuming an underlying binomial distribution) and to adjust for standard demographic and personal risk factors will also be incorporated into the computer package.

MAJOR FINDINGS AND PROPOSED COURSE: (1) The basic mapping and test procedures for the crude North Carolin county rates have been implemented. (2) Residual maps, reflecting the difference between observed rates and the rates that would be expected based on average county estimates of various demographic and personal risk factors, are now being developed.

Once the residual maps are completed, it is proposed that they be compared, if possible, to similar maps generated from individual birth-death certificate data in order to assess the effect of using secondary (or indirect) data instead of primary data to study geographic variability. The results of these comparisons will then be used to evaluate the feasibility of producing maps for the entire country.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Maps of (county-based) indices associated with pregnancy outcomes--particularly maps adjusted for the effects of non-environmental risk factors--could provide valuable leads to the identification of potential health hazards present in man's everyday environment.

PUBLICATIONS

Hogan, M.D., Chi, P-Y., Mitchell, T.J., and Hoel, D.G.: Association between chloroform levels in finished drinking water supplies and various site-specific cancer mortality rates. Journal of Environmental Pathology and Toxicology, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 43002-02 BB																
PERIOD COVERED October 1, 1977 to September 30, 1978																		
TITLE OF PROJECT (80 characters or less) Environmental Contaminants of Infants Foodstuffs																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">W. J. Rogan</td> <td style="width: 35%;">Staff Associate</td> <td style="width: 15%;">BB NIEHS</td> </tr> <tr> <td>Other:</td> <td>B. C. Gladen</td> <td>Staff Fellow</td> <td>BB NIEHS</td> </tr> <tr> <td></td> <td>J. D. McKinney</td> <td>Supervisory Chemist</td> <td>EBCB NIEHS</td> </tr> <tr> <td></td> <td>D. B. Walters</td> <td>Chemist</td> <td>EBCB NIEHS</td> </tr> </table>			PI:	W. J. Rogan	Staff Associate	BB NIEHS	Other:	B. C. Gladen	Staff Fellow	BB NIEHS		J. D. McKinney	Supervisory Chemist	EBCB NIEHS		D. B. Walters	Chemist	EBCB NIEHS
PI:	W. J. Rogan	Staff Associate	BB NIEHS															
Other:	B. C. Gladen	Staff Fellow	BB NIEHS															
	J. D. McKinney	Supervisory Chemist	EBCB NIEHS															
	D. B. Walters	Chemist	EBCB NIEHS															
COOPERATING UNITS (if any) Environmental Biology and Chemistry Branch																		
LAB/BRANCH Biometry Branch																		
SECTION																		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																		
TOTAL MANYEARS: 1.7	PROFESSIONAL: 1.3	OTHER: 0.4																
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER																		
☒ (a1) MINORS ☒ (a2) INTERVIEWS																		
SUMMARY OF WORK (200 words or less - underline keywords) <u>Polychlorinated biphenyl (PCB)</u> contamination of breast milk in the ppb range is well documented, but <u>health effects on infants</u> fed such milk are <u>unstudied</u>. This project involves: (1) <u>establishment of a cohort of breast and formula fed infants</u>; (2) <u>development of methodology to obtain reliable and reproducible samples of human body fluids and tissue from mother and child</u>; (3) <u>development of reliable methods for analysis of PCB's and other related parameters</u>; (4) <u>development and application of statistical procedures for the analysis of data generated from the study</u>; and (5) <u>evaluation of the children for specific outcomes thought to be related to organochlorine exposure due to PCB's</u>.																		

PROJECT DESCRIPTION

METHODS EMPLOYED: The study is a prospective, or follow-up study. Field nurses are hired, trained in protocol administration, and then work at selected hospitals. Subjects are enrolled, informed consent is obtained, and a questionnaire administered to each mother at approximately the time of delivery. Samples of milk, formula, colostrum, placenta, and maternal blood are collected.

These samples are subjected to gas chromatographic and neutron activation analysis for PCB's, DDE(1,1-bis(p-chlorobiphenyl)-2,2-dichloroethane), total organic chlorine (TOCl) and bromine (TOBr), and total soluble organic chlorine (TSOCl) and bromine (TSOBr) in the ppb range.

The children are examined, and follow-up appointments made. Several examinations are performed over the first 6 months of the child's life including 2 behavioral evaluations, one at birth and one at 6 months.

MAJOR FINDINGS AND PROPOSED COURSE: No data analysis is yet complete. Data collection in North Carolina will continue. Major progress to date has been completion of procedures for enrollment of participants and the perfection of techniques required for collection of samples without possibility of contamination. Two contracts have been let for the three North Carolina hospitals scheduled to participate, and several subjects have delivered thus far. Quality assurance procedures for control of chemical analyses and the development of methodology for reliable and reproducible ppb level determination of PCB, DDE, TOCl, TOBr, TSOCl and TSOBr are nearing completion.

The remaining objectives are: (1) to establish the relationship between milk levels, maternal blood levels, cord blood levels, placental levels, and colostrum at birth, and examine the trends in milk concentration over time; (2) to investigate the relationship between PCB and TOCl levels in the neonate and a number of specific outcomes; (3) to follow breast-fed and non breast-fed infants over a period of six months and look for differences in incidence of a number of specific outcomes; (4) to generate other hypotheses about toxic effects of chronic low dose PCB exposure in children; and (5) to establish a cohort of children for follow-up studies.

Plans for long term evaluation of these children are also being made.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The health effects of these low dose environmental pollutants are not well studied in children, and this project should allow identification and quantification of those that occur short term in this group. The methodology for studying such phenomenon is also of interest, and the development of a field efficient method for study of low level pollutants, such as PCB's, in humans is important.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 44002-03 BB								
PERIOD COVERED October 1, 1977 to September 30, 1978										
TITLE OF PROJECT (80 characters or less) Mathematical Population Genetics										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 25%;">PI:</td> <td style="width: 30%;">N. L. Kaplan</td> <td style="width: 30%;">Research Mathematician</td> <td style="width: 15%;">BB NIEHS</td> </tr> <tr> <td>Other:</td> <td>C. H. Langley</td> <td>Section Head</td> <td>LEM NIEHS</td> </tr> </table>			PI:	N. L. Kaplan	Research Mathematician	BB NIEHS	Other:	C. H. Langley	Section Head	LEM NIEHS
PI:	N. L. Kaplan	Research Mathematician	BB NIEHS							
Other:	C. H. Langley	Section Head	LEM NIEHS							
COOPERATING UNITS (if any) Laboratory of Environmental Mutagenesis										
LAB/BRANCH Biometry Branch										
SECTION										
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709										
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0.0								
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS										
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this project is to provide more realistic <u>models</u> for certain phenomenon in <u>population genetics</u>. The work is being conducted in collaboration with scientists in the Laboratory of Environmental Mutagenesis. Current areas of interest include (1) the development of a model which permits estimation of the <u>nucleotide mutation rate</u>, (2) an examination of the "ladder rung" model in order to attempt to compute the probabilities governing the gene frequencies, and (3) the continued development of theoretical areas in <u>stochastic diffusion equations</u> which are applicable to population genetics.										

PROJECT DESCRIPTION

METHODS EMPLOYED: Collaborative research was conducted on problems in population genetics with scientists in the LEM. This research, which augments the population genetics studies performed by the LEM, was probabilistic in nature, emphasizing Markov chain theory and diffusion techniques.

MAJOR FINDINGS AND PROPOSED COURSE: (1) A new model was developed for estimating the nucleotide mutation rate through the use of restrictive enzymes. Since certain approximations were required to perform the computations, an attempt was made to quantify the error introduced by the approximation. (2) Research on the calculation of various probabilities associated with the "ladder rung" model has been initiated. Numerical methods involving the inversion of certain Fourier transforms are being investigated. (3) Some of the diffusion approximations previously derived under this collaborative research effort to predict the effect of autocorrelated random changes in environmental conditions on gene frequency distributions are being re-examined. It may be possible to adapt existing results in the field of stochastic diffusion equations to this problem.

Future work will continue in these areas.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: More realistic models for genetic phenomenon should be beneficial in predicting long-term effects of environmental changes on public health.

PUBLICATIONS

Guess, H.A. and Gillespie, J.H.: Diffusion approximations to linear stochastic difference equations with stationary coefficients. J. Appl. Prob. 14: 58-74, 1977.

Watterson, G.A. and Guess, H.A.: Is the most frequent allele the oldest? Theor. Popn. Biol. 11: 141-160, 1977.

Gillespie, J.H. and Guess, H.A.: The effect of autocorrelation on the progress of natural selection in random environments, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 44003-01 BB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Fetal Wastage and Chemical Exposure		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: S. M. Brown Other: A. K. Bagniewski Medical Officer (Research) Mathematical Statistician BB NIEHS BB NIEHS		
COOPERATING UNITS (if any) Department of Obstetrics, Duke University School of Medicine, Durham, NC		
LAB/BRANCH Biometry Branch		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 2.0	PROFESSIONAL: 2.0	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☒ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The objective is to examine the variations in response of <u>pregnant</u> women when exposed to any of a number of defined environmental <u>mutagens</u> and <u>clastogens</u> . A feasibility pre-study is in progress to test the possibility of using a survey questionnaire instrument or interview in order to assess exposure in occupation, household, personal habits, or other modes. The disease outcomes of interest are <u>spontaneous abortion</u> , or alternatively <u>histopathological</u> or <u>karyological</u> abnormal <u>fetal tissue</u> .		

PROJECT DESCRIPTION

METHODS EMPLOYED: This study is still in the planning phase. At the present time a case control study is envisioned in which one tier is made up of abnormal fetal cases obtained on a hospital basis from menstrual evacuations; and a possible second tier comprised of spontaneous abortion cases is also considered. Stratum-matched controls will be obtained from either or both of the following: normal pregnant women and their fetuses of equal gestational age, matched for appropriate co-variables; and/or cases of surgical abortion of approximately equal gestational age matched for appropriate co-variables. A number of laboratory tests are anticipated on the parents in order to assess presence and degree of mutagen-exposure, abnormal karyology, and related biologic phenomena. Details of the methodology have not yet been resolved.

MAJOR FINDINGS AND PROPOSED COURSE: No findings are currently available from this study. The literature involving spontaneous abortion both in mammals and man has been reviewed. Numerous persons doing research in obstetrics or reproductive physiology in mammals or man have been consulted. The proposed course of the study would be 24-36 months with a multiple hospital base.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The response of man to environmental mutagens, particularly via fetal wastage, may be an important health response not only in itself, but also as an indicator of other more serious and longer range damage to the genome. Nonlethal recessive mutation and malignancy are less obviously manifest but may be reflected when short-term results of this type can be demonstrated with a particular exposure. This methodology may therefore serve as a prototype for *in vivo* studies of mutagen exposure which may have implications for more widespread genetic damage and/or neoplasia.

AMERICAN HEALTH FOUNDATION - New York, New York
(N01 ES 6-2130-02)

TITLE: Species-to-Species Carcinogenesis Extrapolation

CONTRACTOR'S PROJECT DIRECTOR: G. M. Williams, M.D.

PROJECT OFFICER (NIEHS): M. D. Hogan, Ph.D., Mathematical Statistician,
Biometry Branch

DATE CONTRACT INITIATED: June 15, 1976

CURRENT ANNUAL LEVEL: \$13,386

PROJECT DESCRIPTION

OBJECTIVES: To create a data summary from published studies in the field of carcinogenesis and to utilize this data summary to investigate various aspects of the quantification of species differences following exposure to specified carcinogenic agents. These differences will in turn be incorporated into the species-to-species extrapolation process whenever feasible.

METHODS EMPLOYED: All relevant pharmacological and epidemiological literature on a set of agents which have been found to be carcinogenic in man and at least one animal species was reviewed and abstracted.

MAJOR FINDINGS AND PROPOSED COURSE: The principle investigator has reached the following conclusions. (1) There are significant species differences in response to each of the carcinogens evaluated in well-controlled experiments. (2) These differences cannot be reconciled by merely reworking the dose, i.e., by changing the units of measurement in which the dose or dose rate is expressed. (3) Furthermore, for none of the species differences was a metabolic factor identified which could account for them. However, the activated metabolites are difficult or impossible to identify in most cases. Thus, the metabolism studies must be considered insufficient at this point. In addition, none of the metabolism studies was performed specifically to examine species differences in sensitivity. (4) The quantitative data on human exposure is generally inaccurate or non-existent. For the various compounds considered the best data is that associated with DES exposure, and Aflatoxin B₁ is acceptable. However, the data on benzidine is considered inaccurate. (5) Using the best estimate for human exposure, man has the same sensitivity within an order of magnitude as some experimental species.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In the absence of relevant human data, decisions regarding acceptable levels of various environmental agents are increasingly being based on information derived from animal experimentation. One of the major obstacles to the

development of realistic and highly explicit animal models that will accurately predict human response is the problem of species-to-species extrapolation. If we are to significantly reduce our reaction time in responding to environmentally based threats to human health through the vehicle of the animal test system, then it is essential that we successfully confront the quantitative problems involved in species-to-species extrapolation.

FRED HUTCHINSON CANCER RESEARCH CENTER - Seattle, Washington 98104
LOUISIANA TECH UNIVERSITY - Ruston, Louisiana 71272
(RFP #NIH-ES-77-22)

TITLE: Statistical Development of Multistage Carcinogenesis Models

CONTRACTOR'S PROJECT DIRECTORS: Ross L. Prentice, Ph.D. (FHCRC) and
Kenny S. Crump, Ph.D. (LTU)

PROJECT OFFICER (NIEHS): David G. Hoel, Ph.D., Chief, Biometry Branch

DATE CONTRACT INITIATED: May, 1978

CURRENT ANNUAL LEVEL: \$61,491 (FHCRC) and \$46,113 (LTU)

PROJECT DESCRIPTION

OBJECTIVES: To develop and evaluate statistical procedures for utilizing data from animal tests conducted at high, often maximum tolerated dose rates to estimate carcinogenic risks at very low dose rates, such as might be encountered by humans in their daily environment.

MAJOR FINDINGS AND PROPOSED COURSE: Under the FHCRC contract a variety of studies based on proportional hazards and accelerated failure time models will be initiated, including (1) the investigation of various multistage and multipath assumptions leading to tumor incidence rates, (2) the adaptation of the use of cause specific hazard functions and cause specific regression coefficients to the estimation and extrapolation of dose effects in the presence of competing causes of death, and (3) the investigation of the use of censored data generalizations of Wilcoxin and log-rank test statistics in the derivation of estimators of dose effect coefficients.

Under the LTU contract an application of Cox's life table method to the multistage model (for carcinogenesis) will form the basis for extending an existing procedure for analyzing dichotomous data so that time-to-tumor information can be utilized. With this approach hypothesis tests about the shape of the dose response curve at low doses will be developed as well as procedures for obtaining point estimates and confidence intervals for the age-dependent risk of cancer at low dose rates.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In the absence of relevant human data cancer risk estimates extrapolated from laboratory animal experiments will continue to have a major impact on regulatory decision-making policies.

LABORATORY OF BEHAVIORAL AND NEUROLOGICAL TOXICOLOGY

LABORATORY OF BEHAVIORAL AND NEUROLOGICAL TOXICOLOGY

Summary Statement

The scientific efforts of the Laboratory of Behavioral and Neurological Toxicology (LBNT) are directed toward the understanding of the behavioral and neurological effects produced by toxic substances. Special emphasis is placed upon the changes in behavior and neurologic function produced by long-term exposure to low levels of a wide variety of chemical and physical agents present in the environment.

The goals of the Laboratory are (1) to identify laboratory procedures useful in assessing the role of environmental factors in the development of behavioral and neurological abnormalities, (2) determine the mechanism(s) whereby environmental factors produce their behavioral and neurotoxic effects, and (3) identification of conditions which predispose individuals to the behavioral and neurotoxic effects of environmental factors.

The research goals of the Laboratory are supported by both intramural scientists and contracted research. The scope of the overall effort is broad involving national and international programs and adjunct appointments. Training opportunities exist for graduate students at nearby universities, foreign and American postdoctoral scientists, and American scientists on sabbatical leave. Current expertise is in the areas of behavior and neurochemistry.

BEHAVIORAL TOXICOLOGY

The research of this group is devoted largely to the identification of laboratory procedures useful in assessing the effects of environmental factors on behavioral and neurological function and in determining conditions which predispose individuals to the behavioral and neurotoxic effects of these factors.

Group scientists have developed a simple test battery for the neurobehavioral assessment of potential neurotoxins. The tests include gross observations of sensory/motor functioning, hind limb extensor response, forelimb grip strength, startle responsiveness, tremor, performance on an inclined screen, visual placement, rectal temperature, spontaneous motor activity, and measures of acquisition and retention of a learned response. These tests are now being standardized and validated using compounds with known neurotoxic effects.

The effects of polybrominated biphenyl compounds in rats and mice are also being investigated. These compounds have been recognized as an environmental hazard following their inadvertent mixture to animal feed. Rodents exposed to these compounds in their feed show a decrease in body weight, depressed open-field activity, lower forelimb grip strength, and depressed reflexes. An apparent loss of visual placement and the occurrence of hypothermia were noted in some animals. These studies have demonstrated effects of the polybrominated biphenyls

in rodents similar to those reported to occur in humans thus lending some credence to the utility of the test battery. Neurophysiological and neurochemical studies are planned.

Group scientists are also investigating the neurobehavioral toxicity of acrylamide in rats. Acrylamide is a known neurotoxin having effects generally most easily seen as peripheral neuropathy. This compound is being studied in order to more clearly define the validity of the simple test battery. As expected, deficits in hind limb function have been found with doses which do not affect forelimb strength further illustrating the usefulness of the two test procedures developed to measure these functions.

Several studies are in progress or being initiated concerning the neurobehavioral toxicity of agents administered prenatally, neonatally, or both. These studies are being conducted in collaboration with other laboratories at NIEHS and/or the local universities. One such project concerns the neurobehavioral toxicity of lead when administered neonatally. Another study seeks to determine the neurobehavioral effects of prenatal exposure to 3,4,3',4'-tetrachlorobiphenyl in mice. Offspring of mothers given the compound on days 6-13 of gestation are being assessed for neurobehavioral deficits for up to 1 year after birth. Another project concerns the effects of neonatal exposure to diethylstilbestrol and testosterone on the neurobehavioral functioning of rats at various times during development. Two other studies concern the long-term neurobehavioral effects of perinatal exposure of Japanese Quail eggs (*Coturnix coturnix japonica*) to microwave irradiation or 2,4-diphenoxycetic acid. Measures being studied are sexual and reproductive behaviors, a battery of behavioral and neurological measurements, learning and retention, and baseline performance on a multiple fixed ratio/fixed interval schedule.

NEUROCHEMISTRY

The primary goal of the neurochemistry workgroup is concerned with determining the biochemical bases for the effects of environmental agents on behavior and neurological function. To date the major focus of attention has been on some of the consequences of modulation of gonadal steroid production in the neonate with respect to sexual differentiation in rat brain. Monoamine oxidase levels and turnover rates are being used as one criterion for reflection of sex steroid modulation. Other criteria include estimations of serotonin and catecholamine levels and turnover rates in various parts of the brain. The results of these studies are being correlated with alterations in behaviors of the animals.

PERSONNEL

Additions to the Laboratory were: Staff Fellow - R. E. Squibb; Technician - W. C. Byrd.

Losses from the Laboratory were: Intergovernment Personnel Act - E. A. Zeller.

OTHER ACTIVITIES

Dr. P. A. Cabe: Adjunct Assistant Professor, Department of Psychology, University of North Carolina, lectures presented to psychology students of the

University of North Carolina; Invited speaker at a symposium entitled "First Encounters of a Pictorial Kind" sponsored by the American Psychological Association.

Dr. C. L. Mitchell: Adjunct Professor, Department of Pharmacology, University of North Carolina; lectures presented to nursing students of the University of North Carolina; Member, Intramural Council, NIEHS; Member, NIEHS Task Force II Program Planning Group; Councilor, North Carolina Society for Neuroscience; Organizer, Conference on Target Organ Toxicity: Nervous System (Behavioral and Neurotoxicity); Organizer, Panel on Behavioral and Neurotoxic Effects of Environmental Agents, Winter Conference on Brain Research; Invited speaker, Workshop on Biological Monitoring, sponsored by the State University of North Carolina; Invited seminar entitled "A Behavioral Toxicological Testing Battery and Its Validation" presented at the Institute of Toxicology, University of Zurich, Zurich, Switzerland; Invited seminar entitled "Behavioral and Neurological Toxicology," Laboratory of Neuropsychology, National Institute of Mental Health; Participant in the US-USSR Medical Science and Public Health Agreement and the US-USSR Agreement on Cooperation in the Field of Environmental Protection.

Dr. H. A. Tilson: Invited participant, Workshop on Polybrominated Biphenyls, sponsored by Michigan State University; Invited participant, Workshop on Behavioral and Neurotoxic Effects of Environmental Agents, Winter Conference on Brain Research; Invited paper entitled "Development of Methods for the Assessment of Behavioral and Neurological Toxicities in Adult Animals" presented at Gordon Research Conference on Toxicology and Safety Evaluations.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70220-05 LBNT																	
PERIOD COVERED October 1, 1977 to September 30, 1978																			
TITLE OF PROJECT (80 characters or less) Neurobehavioral Toxicity of Developmental Exposure to Lead																			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																			
<table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 35%;">C. A. Kimmel</td> <td style="width: 40%;">Senior Staff Fellow</td> <td style="width: 15%;">LBNT NIEHS</td> </tr> <tr> <td rowspan="4">Other:</td> <td>J. S. Woods</td> <td>Head, Biochemical Toxicology Group</td> <td>LET NIEHS</td> </tr> <tr> <td>B. A. Fowler</td> <td>Research Biologist</td> <td>LET NIEHS</td> </tr> <tr> <td>E. E. McConnell</td> <td>Veterinary Pathologist</td> <td>EBCB NIEHS</td> </tr> <tr> <td>T. Damstra</td> <td>Biochemist</td> <td>OHHA NIEHS</td> </tr> </table>			PI:	C. A. Kimmel	Senior Staff Fellow	LBNT NIEHS	Other:	J. S. Woods	Head, Biochemical Toxicology Group	LET NIEHS	B. A. Fowler	Research Biologist	LET NIEHS	E. E. McConnell	Veterinary Pathologist	EBCB NIEHS	T. Damstra	Biochemist	OHHA NIEHS
PI:	C. A. Kimmel	Senior Staff Fellow	LBNT NIEHS																
Other:	J. S. Woods	Head, Biochemical Toxicology Group	LET NIEHS																
	B. A. Fowler	Research Biologist	LET NIEHS																
	E. E. McConnell	Veterinary Pathologist	EBCB NIEHS																
	T. Damstra	Biochemist	OHHA NIEHS																
COOPERATING UNITS (if any) Child Development Institute of Biological Science, UNC Laboratory of Environmental Toxicology, NIEHS Office of Health Hazards Environmental Biology and Chemistry Branch, NIEHS Assessment, NIEHS																			
LAB/BRANCH Laboratory of Behavioral and Neurological Toxicology																			
SECTION Behavioral Toxicology Group																			
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																			
TOTAL MANYEARS: 2	PROFESSIONAL: 2	OTHER: 0																	
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																			
SUMMARY OF WORK (200 words or less - underline keywords) The <u>developmental</u> effects of <u>lead</u> are being studied using an <u>interdisciplinary</u> approach to evaluate and compare various toxicological parameters with an emphasis on <u>neurobehavioral</u> measurements. A broad battery of observations focussed on <u>physical</u> and <u>reflex</u> development, and <u>behavioral</u> functions, is being employed to determine the <u>neurotoxic</u> potential of lead. These observations are being related to <u>teratogenicity</u>, <u>body burden</u> of lead, <u>histopathologic</u> and <u>ultrastructural</u> changes, effects on the <u>biochemistry</u> of target tissues (brain and kidney), as well as <u>functional</u> changes in other systems. This multidisciplinary approach will be evaluated in terms of its usefulness for routine assessment of compounds with unknown neurotoxic potential.																			

PROJECT DESCRIPTION

METHODS EMPLOYED: Female rats obtained at weaning are being exposed to the following doses of lead (as lead acetate) in deionized drinking water: 0, 0.5, 5, 25, 50, and 250 ppm. Exposure is continued for 6-7 weeks at which time animals are mated; then exposure continues through pregnancy, lactation, and subsequently to the offspring following weaning. At 21 days of gestation some animals are sacrificed and a routine teratology exam is performed. Other animals are sacrificed at various times throughout the exposure period and blood, brain, and bone collected for analysis of lead by atomic absorption spectroscopy. Another group of animals is allowed to litter, and offspring are examined for physical milestones (e.g., eye opening, pinna detachment), reflex ontogeny (e.g., righting, visual placing), and later for locomotor coordination, activity, learning acquisition of several responses, and sexual behavior. Additional evaluations include (1) measurement of brain acetyl- and butyrylcholinesterase in neonates and weanlings, (2) evaluation of reproductive function in mated offspring, and (3) evaluation of immunologic competence in the young (Project No. Z01 ES 30007-05 EBCB). Offspring are exposed until 9 months of age at which time they are sacrificed and blood samples are taken for lead analysis, routine hematologic examination and clinical chemistries. Body and organ weights are obtained; tissues are taken for histopathology and electron microscopy; and kidneys are homogenized and used to study heme biosynthesis and mitochondrial respiratory activity.

MAJOR FINDINGS AND PROPOSED COURSE: Lead doses of 50 and 250 ppm produce overt toxic effects on body weight, physical and behavioral development, and biochemical and morphologic parameters. At 25 ppm, there is an effect on time of vaginal opening in parent and F₁ females and in the immunologic competence. In 9-month-old male rats, there is a significant effect on kidney weight at all doses and on changes in renal morphology starting at the 5 ppm dose level. These changes in the kidney are the most subtle effects due to lead observed so far. Behavioral testing of animals exposed to 250 ppm of lead up to 6-9 months of age indicated impaired surface righting responses and slightly delayed maturation of locomotor response patterns. Significant delays in the development of sensorimotor reflexes were not noted. Animals exposed to 0-50 ppm of lead showed no impaired ability to learn and perform operant schedules of reinforcement although animals receiving 50 ppm appeared to show decreased resistance to extinction. In the acquisition and performance of a food reinforced position discrimination task in a T maze, chronic exposure to 5-50 ppm of lead resulted in a dose-related impairment. However, animals receiving 250 ppm were affected less than the rats receiving 50 ppm. Acquisition of a two-way shuttle box avoidance task was generally impaired in animals receiving 5-50 ppm of lead and the effect may have been dependent upon the age of the subject at the time of testing. Evaluation of female and male reproductive behaviors or maternal responses in lead treated animals did not reveal statistically reliable neurobehavioral deficits.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: There is an immediate need for the study and evaluation of toxicant effects on

behavior and the assessment of such effects as early indicators of toxicity. These studies relate directly to the tremendous problems of congenital mental retardation and abnormal behavior in children for which there is, in most cases, no known etiology. In terms of relevance to the field of toxicology, many questions are being addressed in this project which relate to sensitivity, approach, and validity of presently used toxicological evaluations.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70800-02 LBNT																
PERIOD COVERED October 1, 1977 to September 30, 1978																		
TITLE OF PROJECT (80 characters or less) Neurochemical Correlates of Behavioral Toxicology																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 35%;">W. E. Wilson</td> <td style="width: 40%;">Research Chemist</td> <td style="width: 15%;">LBNT NIEHS</td> </tr> <tr> <td>Other:</td> <td>A. K. Agrawal</td> <td>Visiting Fellow</td> <td>LBNT NIEHS</td> </tr> <tr> <td></td> <td>C. L. Mitchell</td> <td>Laboratory Chief</td> <td>LBNT NIEHS</td> </tr> <tr> <td></td> <td>C. A. Lamartiniere</td> <td>Senior Staff Fellow</td> <td>LET NIEHS</td> </tr> </table>			PI:	W. E. Wilson	Research Chemist	LBNT NIEHS	Other:	A. K. Agrawal	Visiting Fellow	LBNT NIEHS		C. L. Mitchell	Laboratory Chief	LBNT NIEHS		C. A. Lamartiniere	Senior Staff Fellow	LET NIEHS
PI:	W. E. Wilson	Research Chemist	LBNT NIEHS															
Other:	A. K. Agrawal	Visiting Fellow	LBNT NIEHS															
	C. L. Mitchell	Laboratory Chief	LBNT NIEHS															
	C. A. Lamartiniere	Senior Staff Fellow	LET NIEHS															
COOPERATING UNITS (if any) Laboratory of Environmental Toxicology, NIEHS																		
LAB/BRANCH Laboratory of Behavioral and Neurological Toxicology																		
SECTION Neurochemistry Workgroup																		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																		
TOTAL MANYEARS: 2.3	PROFESSIONAL: 2.3	OTHER: 																
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																		
SUMMARY OF WORK (200 words or less - underline keywords) In our investigations concerned with the <u>biochemical bases for the effects of environmental agents on animal behavior</u>, we are focusing our attention on some of the consequences of <u>modulation of gonadal steroid production in the neonate</u> with respect to <u>sexual differentiation in rodent (rat) brain</u>. <u>Monoamine oxidase (MAO) levels and turnover rates</u> will be used as one criterion for reflection of <u>sex steroid modulation</u>, while other criteria will include estimations of <u>serotonin and catecholamine levels and turnover rates</u> in various parts of the brain. The results of these biochemical studies will be interpreted in the context of alterations in behaviors of the experimental animals.																		

PROJECT DESCRIPTION

METHODS EMPLOYED: Biochemical measurements as well as surgical and dissection techniques will generally be performed according to published techniques with necessary adaptations. Behavioral testing will be performed according to designs constructed by H. Tilson, P. Cabe, and C. Mitchell.

MAJOR FINDINGS AND PROPOSED COURSE: Our prior studies, on rats which were administered clorgyline and/or deprenyl, indicated that there was no significant correlation between Amex motor activity and the extent of inhibition of monoamine oxidase (MAO) types A and/or B in homogenates prepared from whole brain of the experimental animals.

In a collaborative study, with C. Lamartiniere, it was observed that castration of newborn rats on postnatal day 1 led to the following situation on day 63: (a) Whole brain homogenate levels of MAO types A and B were diminished relative to control values, whereas, the activities of MAO and of 5 of 6 other enzymes in liver homogenates of the treated animals did not differ from control values. (b) Amex motor activities of castrated males was similar to that of control females rather than control males one and two days before sacrifice. (c) Administration of diethylstilbestrol (DES) within one week after castration did not affect MAO activity but did seem to enhance female-like Amex motor activity of treated rats. (d) Administration of testosterone propionate (TP) within one week after castration brought the levels of MAO activity up to control levels and tended to make the Amex motor behavior more like that of control males.

While there appeared to be no causal relationship between Amex motor activity and the extent to which whole brain homogenate MAO activities had been titrated in vivo with clorgyline or with deprenyl, the influence of modification of sex steroid exposure of the neonate obviously extended to the behavioral as well as the neurochemical level.

The influence of TP on levels and on turnover rats of MAO types A and B will be investigated to ascertain whether TP affects the behavior of either type in a selective fashion similar to that reported for estrogen enhancement of MAO type A turnover.

In following up our initial observations, we propose to try to obtain answers to the following questions: (a) Are variations in MAO levels in 9 week old rats confined to small parts of the brain, such as the hypothalamus, or are they general? (2) Can the doses of TP and of DES be optimized with respect to their effects on MAO levels and on behavior? (3) Can tests other than Amex motor behavior provide more sensitive and/or discriminatory correlates for the consequences of modulation of sexual differentiation of the brain of the neonatal rat? (4) Can other biochemical and/or morphological correlates of steroid regulation of neonatal brain differentiation be recognized (eg., can prior reports relative to serotonin levels in neonatal brain on day 12 be confirmed in our animals and can such observations be correlated with alterations in neurotransmitter turnover? (5) Can responses to the antiandrogen, cyproterone

be correlated with those to castration in the male rat. Cyproterone, a known teratogen (it interferes with male reproductive tract differentiation), may prove to be useful in connection with studies involving DES effects as well as testosterone effects on behavior.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Alterations in local levels of and rates of turnover of biogenic amines and other putative neurotransmitter compounds are frequently associated with neurotoxic and behavioral effects of drugs and of environmental agents. Furthermore, clinical and pharmacological experience have indicated that the modulation of various transmitter metabolizing, storage, and/or transporting systems frequently lead to significant alterations in the affective state and to neurologic and psychiatric disorders.

Numerous reports have suggested that levels of monoamine oxidase in various parts of the brain or of other tissues may be of significance in signaling perturbations in regulation of local levels of biogenic amines. Levels of MAO, which vary with age and sex in brain of humans and experimental animals, are influenced by sex steroids as well as by compounds or agents of environmental significance (eg., DES, pesticides, etc.). Our efforts to determine whether and to what extent turnover of neurotransmitters and local levels of neurotransmitter metabolizing enzymes are influenced by modulation of the sex steroid levels in the neonate will be of importance in interpreting the biochemical bases of resulting behavioral alterations in treated or exposed animals.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70805-02 LBNT																				
PERIOD COVERED October 1, 1977 to January 1, 1978																						
TITLE OF PROJECT (80 characters or less) Neurotoxicological Effects of Polybrominated Biphenyls in Rats and Mice																						
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																						
<table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 40%;">H. A. Tilton</td> <td style="width: 40%;">Head, Behavioral Toxicology Group</td> <td style="width: 10%;">LBNT NIEHS</td> </tr> <tr> <td>Other:</td> <td>P. A. Cabe</td> <td>Senior Staff Fellow</td> <td>LBNT NIEHS</td> </tr> <tr> <td></td> <td>D. Mitchell</td> <td>Staff Fellow</td> <td>LBNT NIEHS</td> </tr> <tr> <td></td> <td>J. A. Moore</td> <td>Associate Director</td> <td>RRP NIEHS</td> </tr> <tr> <td></td> <td>C. L. Mitchell</td> <td>Laboratory Chief</td> <td>LBNT NIEHS</td> </tr> </table>			PI:	H. A. Tilton	Head, Behavioral Toxicology Group	LBNT NIEHS	Other:	P. A. Cabe	Senior Staff Fellow	LBNT NIEHS		D. Mitchell	Staff Fellow	LBNT NIEHS		J. A. Moore	Associate Director	RRP NIEHS		C. L. Mitchell	Laboratory Chief	LBNT NIEHS
PI:	H. A. Tilton	Head, Behavioral Toxicology Group	LBNT NIEHS																			
Other:	P. A. Cabe	Senior Staff Fellow	LBNT NIEHS																			
	D. Mitchell	Staff Fellow	LBNT NIEHS																			
	J. A. Moore	Associate Director	RRP NIEHS																			
	C. L. Mitchell	Laboratory Chief	LBNT NIEHS																			
COOPERATING UNITS (if any) Research Resources Program, NIEHS																						
LAB/BRANCH Laboratory of Behavioral and Neurological Toxicology																						
SECTION Behavioral Toxicology Group																						
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																						
TOTAL MANYEARS: 0.40	PROFESSIONAL: 0.20	OTHER: 0.20																				
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																						
SUMMARY OF WORK (200 words or less - underline keywords) This is a completion report of work that was in progress at the time of the last reporting. Polybrominated biphenyls (PBB) have been recognized as an environmental hazard following the inadvertent mixture of PBB to animal feed. Rats and mice were tested after 30 days of dosing by gavage with Firemaster FF-1 (0.03-30 mg/kg), a commercial PBB-containing mixture, with <u>hexabromobiphenyl</u> (HBB; 0.168-16.8 mg/kg), the major PBB component, or with corn oil vehicle, and 30 days after dosing ceased. Effects observed included decreases in <u>body weights</u> , <u>open-field activity</u> , <u>forelimb grip strength</u> , and <u>muscular reflexes</u> . <u>Loss of visual placement and hypothermia</u> were seen in some animals. FF-1 was generally more potent than HBB. Rats were more affected than mice. Rats did not recover as well (or worsened) relative to mice during post-dosing. The results suggest neurobehavioral deficits may be produced by PBB at levels lower than those which produce clinical signs of toxicity.																						

PROJECT DESCRIPTION

METHODS EMPLOYED: Male and female rats and mice were dosed with Firemaster FF-1, a commercial PBB mixture, (0.03, 3.0, and 30 mg/kg) or with 2,4,5,2',4'5'-hexabromobiphenyl (HBB; 0.168, 1.68, 16.8 mg/kg) or corn oil vehicle. HBB is the major PBB component of Firemaster FF-1; doses of FF-1 and HBB were equimolar for HBB. Doses were administered per os 5 days/week for 30 days. Testing with a battery of procedures intended to evaluate a wide range of possible deficits was then administered. A second administration of the battery occurred 30 days later, 30 days after dosing ceased. The measures included: body weight, sensorimotor reflexes (corneal reflex, pinna reflex, toe and tail pinch responses), visual placement, forelimb grip strength, open field activity, emotionality (open field defecations and urinations), and rectal temperature.

MAJOR FINDINGS AND PROPOSED COURSE: Relative to controls, dosed animals showed decreased body weights, depressed open-field activity, lower forelimb grip strength, and depressed reflexes. An apparent loss of visual placement and the occurrence of hypothermia were noted in some animals. Animals dosed with FF-1 were generally more affected than those dosed with HBB, and rats seemed to be more affected than mice. Mice showed more post-dosing recovery than rats. Rats, in fact, appeared to have deteriorated somewhat in the post-dosing period. No obvious overt signs of toxicity were observed. Thus, PBB may produce neurobehavioral effects at doses lower than those which yield clinical signs of toxicosis.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This work is related to the program of the Institute in that it provided basic research into an animal model of human environmental disease. It is significant for biomedical research in that it was demonstrated that the animal model could provide 'early warning' in the sense that the neurobehavioral tests differentiated dosed from undosed animals, in two species, before signs of clinical toxicosis could be seen.

PUBLICATIONS

Tilson, H. A., Cabe, P. A., and Mitchell, C. L.: Behavioral and neurological toxicity of polybrominated biphenyls in rats and mice. Envir. Health Perspec. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70810-02 LBNT												
PERIOD COVERED October 1, 1977 to September 30, 1978														
TITLE OF PROJECT (80 characters or less) The Etiology of Pica in the Rat														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">R. E. Squibb</td> <td style="width: 35%;">Staff Fellow</td> <td style="width: 15%;">LBNT NIEHS</td> </tr> <tr> <td>Other:</td> <td>D. Mitchell</td> <td>Staff Fellow</td> <td>LBNT NIEHS</td> </tr> <tr> <td></td> <td>H. A. Tilson</td> <td>Head, Behavioral Toxicology Group</td> <td>LBNT NIEHS</td> </tr> </table>			PI:	R. E. Squibb	Staff Fellow	LBNT NIEHS	Other:	D. Mitchell	Staff Fellow	LBNT NIEHS		H. A. Tilson	Head, Behavioral Toxicology Group	LBNT NIEHS
PI:	R. E. Squibb	Staff Fellow	LBNT NIEHS											
Other:	D. Mitchell	Staff Fellow	LBNT NIEHS											
	H. A. Tilson	Head, Behavioral Toxicology Group	LBNT NIEHS											
COOPERATING UNITS (if any) Department of Psychology, University of Southern California														
LAB/BRANCH Laboratory of Behavioral and Neurological Toxicology														
SECTION Behavioral Toxicology Group														
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709														
TOTAL MANYEARS: 0.60	PROFESSIONAL: 0.30	OTHER: 0.30												
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords) <u>A continuing series of experiments seeks to validate the functional utility of pica (geophagia) as a behavioral assay of toxicosis in the rat.</u> The present studies sought to demonstrate the effects of orally administered <u>lithium chloride</u>, <u>apomorphine</u>, and <u>acrylamide</u> on the kaolin consumption of <u>Fischer strain albino rats</u>. Other substances will be studied in future experiments.														

PROJECT DESCRIPTION

METHODS EMPLOYED: Male rats of the Charles River CDF 344 strain are individually housed with a 12 hr light dark cycle and provided food (NIH #31 diet), water, and kaolin ad libitum. Baseline data for body weights, food, water, and kaolin consumption are collected daily for at least 10 days. The rats are dosed with vehicle three times a week for two weeks and then dosed with a series of repeated exposures to 127.2 mg/kg lithium chloride in one experiment and 0, 0.25, 0.50, and 1.00 mg/kg of apomorphine in another.

MAJOR FINDINGS AND PROPOSED COURSE: So far it has been determined that Charles River CDF 344 rats dosed with 127.2 mg/kg of lithium chloride at dose volumes of 1 ml/kg and 20 ml/kg body weight administered either intraperitoneally or by gavage engaged in geophagia after the second dose. Rats with the 20 ml/kg volume and ip mode of administration, however, did not. In this study, increased water consumption seemed to be the more sensitive indicator of lithium toxicosis.

In a comparable study in which 0, 0.25, 0.50, and 1.0 mg/kg of apomorphine was administered ip at a volume of 1 ml/kg on four consecutive occasions, there was no evidence of geophagia across treatment groups. In a third experiment, still on-going at this time, kaolin consumption and/or geophagia to acrylamide administration will be determined. Future dose ranging studies with other substances are also planned so that the usefulness of geophagia as an assay for toxicosis can be more fully determined.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The development of a geophagia model to assay toxicosis behaviorally will serve to clarify the etiology of pica and will also provide a method whereby behavioral and biochemical mechanisms of toxicosis can be examined.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90000-01 LBNT												
PERIOD COVERED January 1, 1978 to July 1, 1978														
TITLE OF PROJECT (80 characters or less) Toxicity of Selenium, Alone and in Combination with Silver, in Rats														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT														
<table> <tr> <td>PI:</td> <td>P. A. Cabe</td> <td>Senior Staff Fellow</td> <td>LBNT NIEHS</td> </tr> <tr> <td>Other:</td> <td>N. Carmichael</td> <td>Visiting Fellow</td> <td>LET NIEHS</td> </tr> <tr> <td></td> <td>H. A. Tilson</td> <td>Head, Behavioral Toxicology Group</td> <td>LBNT NIEHS</td> </tr> </table>			PI:	P. A. Cabe	Senior Staff Fellow	LBNT NIEHS	Other:	N. Carmichael	Visiting Fellow	LET NIEHS		H. A. Tilson	Head, Behavioral Toxicology Group	LBNT NIEHS
PI:	P. A. Cabe	Senior Staff Fellow	LBNT NIEHS											
Other:	N. Carmichael	Visiting Fellow	LET NIEHS											
	H. A. Tilson	Head, Behavioral Toxicology Group	LBNT NIEHS											
COOPERATING UNITS (if any) Laboratory of Environmental Toxicology, NIEHS														
LAB/BRANCH Laboratory of Behavioral and Neurological Toxicology														
SECTION Behavioral Toxicology Group														
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709														
TOTAL MANYEARS: 0.60	PROFESSIONAL: 0.20	OTHER: 0.40												
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords) The present work looked for a <u>detoxification</u> interaction between <u>selenium</u> and a relatively non-toxic metal, <u>silver in rats</u> . Since metals, including selenium, have been reported to have neurotoxic effects, a limited set of neurobehavioral measures was also taken, concentrating primarily on motor strength and reflex measures. Body weight decreases occurred in the group dosed with selenium, though gross <u>food consumption</u> was elevated. <u>Water consumption</u> was decreased in all dosed groups. Transient effects were seen in the silver and silver/selenium groups in <u>hind foot posture</u> (foot drop) and in the selenium-dosed groups on <u>fore-limb grip strength</u> and <u>spontaneous activity</u> . The pattern of results, however, does not allow an inference of unequivocal neurotoxicity for selenium under the conditions employed. The fact that body weight differences occurred in the selenium alone but not the silver alone or silver/selenium group argues for a protective effect of silver against some general health effects of selenium.														

PROJECT DESCRIPTION

METHODS EMPLOYED: Rats were dosed in drinking water for a total of 12 weeks with silver (1000 ppm of elemental silver as silver nitrate), selenium (10 ppm of elemental selenium as sodium selenate) or a mixture of these two solutions. Body weights and gross food and water consumptions were taken weekly. Neuro-behavioral measures were taken biweekly. These included tests of caudal motor reflexes (tail tip curl reflex, hind limb posture/foot drop), forelimb grip strength, hind limb strength, and inclined screen coordination. Spontaneous motor activity measures were taken in the last three test sessions.

MAJOR FINDINGS AND PROPOSED COURSE: Body weights for the selenium alone group were depressed from the third week of dosing on, although food consumption (g food/g body weight/day) was elevated for those animals. Food consumption for the silver/selenium group was also typically elevated though not as markedly. Water consumption (ml water/g body weight/day) was decreased consistently for all dosed animals. That body weights for the silver/selenium and silver alone groups never differed from controls suggests that silver does have some protective effect against selenium toxicity. The motor function tests showed no consistent pattern, although when (transient) differences from controls occurred they were more likely to be in the selenium alone group than any other. It would be difficult to conclude that selenium produced a neurotoxic effect under the test conditions used. Further research, however, with a more extensive battery of tests would appear to be warranted.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Selenium in excess is known to have effects on human health. Its putative detoxifying effect with other metals, especially given its demonstrated nutritional essentiality, suggests a possible therapeutic mechanism in metal intoxication. The possibility of a role for selenium in human metal toxicity therapy places this research within the scope of the Institute's program.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90001-01 LBNT																
PERIOD COVERED October 1, 1977 to September 30, 1978																		
TITLE OF PROJECT (80 characters or less) Effects of Acrylamide on Food, Kaolin, and Water Consumption and Spontaneous Activity of Rats																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 10%; vertical-align: top;">PI:</td> <td style="width: 30%;">R. E. Squibb</td> <td style="width: 40%;">Staff Fellow</td> <td style="width: 20%;">LBNT NIEHS</td> </tr> <tr> <td style="vertical-align: top;">Other:</td> <td>H. A. Tilson</td> <td>Head, Behavioral Toxicology Group</td> <td>LBNT NIEHS</td> </tr> <tr> <td></td> <td>C. L. Mitchell</td> <td>Laboratory Chief</td> <td>LBNT NIEHS</td> </tr> <tr> <td></td> <td>D. Mitchell</td> <td>Staff Fellow</td> <td>LBNT NIEHS</td> </tr> </table>			PI:	R. E. Squibb	Staff Fellow	LBNT NIEHS	Other:	H. A. Tilson	Head, Behavioral Toxicology Group	LBNT NIEHS		C. L. Mitchell	Laboratory Chief	LBNT NIEHS		D. Mitchell	Staff Fellow	LBNT NIEHS
PI:	R. E. Squibb	Staff Fellow	LBNT NIEHS															
Other:	H. A. Tilson	Head, Behavioral Toxicology Group	LBNT NIEHS															
	C. L. Mitchell	Laboratory Chief	LBNT NIEHS															
	D. Mitchell	Staff Fellow	LBNT NIEHS															
COOPERATING UNITS (if any) Duke University Department of Psychology, University of Southern California																		
LAB/BRANCH Laboratory of Behavioral and Neurological Toxicology																		
SECTION Behavioral Toxicology Group																		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																		
TOTAL MANYEARS: 0.75	PROFESSIONAL: 0.25	OTHER: 0.50																
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																		
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of these studies is to determine the effects of chronic exposure to <u>acrylamide on food and water consumption</u> and spontaneous locomotor activity in <u>domiciliary cages</u>. Attempts will be made to measure the changes in each behavioral variable as a function of dose and length of exposure and to associate behavioral dysfunction with <u>neurophysiological</u> and <u>neurochemical</u> measures.																		

PROJECT DESCRIPTION

METHODS EMPLOYED: Adult male, Fischer strain rats are housed individually in stainless steel home cages. Measures of food, kaolin, and water ingestion, as well as home cage crossings, are obtained on-line using a laboratory computer system. Frequency of ingestive responses and locomotor activity and conditional probabilities of each response relative to all others are determined for each subject during consecutive 2 hour time periods. Daily (24 hr) measurements of the amount of food, water, and kaolin ingested, the number of activity counts and body weights are also obtained.

The effects of acrylamide given orally 3 times per week on baseline domiciliary behaviors are currently being studied. The time and cumulative dose required to observe behavioral toxicity are being assessed. Eventually, studies to evaluate the effects of acrylamide on nerve conduction velocities, amplitude, and duration of axonal firing and muscle strength will be studied in behaviorally affected rats. In addition, the effects of acrylamide on the turnover and disposition of brain neurotransmitters, such as dopamine and norepinephrine, in various regions of the nervous system will all determined.

MAJOR FINDINGS AND PROPOSED COURSE: These experiments are currently underway and fully analyzed data are not yet available.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies will attempt to identify subtle behavioral changes occurring during chronic exposure to low levels of acrylamide and to correlate those changes with neurophysiological and neurochemical alterations. The results of these studies may lead to the identification of highly sensitive bioassays for the assessment of neurotoxicity and will prove useful in the study of the mechanism of action of agents that produce 'dying-back' polyneuropathies.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90002-01 LBNT						
PERIOD COVERED March 1, 1978 to September 30, 1978								
TITLE OF PROJECT (80 characters or less) Effects of Diet and Food Restriction on Acrylamide Toxicity in Rats								
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%;"> <tr> <td style="width: 33%;">PI: R. E. Squibb</td> <td style="width: 33%;">Staff Fellow</td> <td style="width: 33%;">LBNT NIEHS</td> </tr> <tr> <td>Other: H. A. Tilson</td> <td>Head, Behavioral Toxicology Group</td> <td>LBNT NIEHS</td> </tr> </table>			PI: R. E. Squibb	Staff Fellow	LBNT NIEHS	Other: H. A. Tilson	Head, Behavioral Toxicology Group	LBNT NIEHS
PI: R. E. Squibb	Staff Fellow	LBNT NIEHS						
Other: H. A. Tilson	Head, Behavioral Toxicology Group	LBNT NIEHS						
COOPERATING UNITS (if any)								
LAB/BRANCH Laboratory of Behavioral and Neurological Toxicology								
SECTION Behavioral Toxicology Group								
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709								
TOTAL MANYEARS: 0.40	PROFESSIONAL: 0.20	OTHER: 0.20						
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS								
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of these experiments is to determine the effects of two commonly used diets and <u>food restriction</u> on the <u>neurobehavioral toxicity</u> of <u>acrylamide</u> in rats. Changes in <u>activity wheel responses</u> as a function of cumulative <u>dose</u> and <u>duration</u> of exposure to acrylamide, diet, and food restriction will be studied.								

PROJECT DESCRIPTION

METHODS EMPLOYED: Weanling male, albino Fischer strain rats are maintained in standard activity wheels. Daily baseline measures of activity wheel turns, food and water ingestion, and body weights are established in animals fed a purified (Purina 5755) or a cereal-based (NIH #31) diet. The effects of diet on the neurotoxic effects of 0-20 mg/kg of acrylamide on activity wheel turns and consumption measures are being studied over a period of 90 days. In a related study, rats maintained on natural or purified diets will be chronically exposed to acrylamide and then restricted at various points to 5 g food/rat/day for various amounts of time. Alterations in food restriction-induced hypermotility by exposure to various doses of acrylamide will be determined.

MAJOR FINDINGS AND PROPOSED COURSE: This study is in progress and results are not yet available.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM AT THE INSTITUTE: The effects of diet and nutrition on the manifestation of neurotoxicity has not been studied systematically, although it has obvious importance. Results from the diet experiment may provide insight into the possible role of nutritional factors (impaired vitamin or other nutrient usage or availability) on the mechanism of action of acrylamide toxicity.

The second experiment involving food restriction procedures may yield information concerning appropriate techniques for the unmasking of neurotoxicities in animals chronically dosed with agents such as acrylamide.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90003-01 LBNT
PERIOD COVERED January 1, 1978 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Effects of Developmental Exposure to Polychlorinated Biphenyls on Neuro-behavioral Functioning of Mice		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: H. A. Tilson Head, Behavioral Toxicology Group LBNT NIEHS Other: G. W. Lucier Head, Developmental Toxicology Group LET NIEHS		
COOPERATING UNITS (if any) Laboratory of Environmental Toxicology, NIEHS Endocrine Labs of Madison, Madison, Wisconsin		
LAB/BRANCH Laboratory of Behavioral and Neurological Toxicology		
SECTION Behavioral Toxicology Group		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.30	PROFESSIONAL: 0.10	OTHER: 0.20
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of these experiments is to determine the neurobehavioral effects of prenatal exposure to 3,4,3',4'-tetrachlorobiphenyl (PCBs) in mice. Offspring of mothers given PCBs on days 6-13 of gestation are being assessed for neurobehavioral deficits for up to <u>1 year</u> after birth.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Pregnant mice are given 3,4,3',4'-tetrachlorobiphenyl by gavage on days 6-13 of gestation. The PCB-exposed offspring are assessed for the presence or absence of rapid circular movements or "spinning" at a time prior to weaning. At 21 days of age, spinning and non-spinning mice are selected with control mice for evaluation in a longitudinal behavioral study. The measures include: exploratory activity, rectal temperature, forelimb grip strength, visual placement, ability to balance on a wire, acquisition of one-way avoidance response, and body weights. Tests are conducted at 1, 2, 6, and 12 months of age. In addition, diurnal activity of mice housed in groups of four is assessed over a 4-5 day period following each neurobehavioral assessment.

MAJOR FINDINGS AND PROPOSED COURSE: These experiments are still underway. In addition to the above neurobehavioral profile, future studies will include measures of food and water consumption, a systematic study of environmental factors that elicit "spinning" behavior, and psychopharmacological studies of possible homeostatic changes in catecholaminergic and cholinergic functioning in the central nervous system.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The behavioral teratogenic potential of PCBs have not been assessed systematically. The experiments described above will provide a long range evaluation of perinatal exposure to PCBs on neurobehavioral functioning. Some insight into the mechanism by which perinatally administered PCBs affects the nervous system may also be attained.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90004-01 LBNT
PERIOD COVERED December 15, 1977 to July 1, 1978		
TITLE OF PROJECT (80 characters or less) Neurobehavioral Assessment of Rats Given Carbon Disulfide		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: H. A. Tilson Head, Behavioral Toxicology Group LBNT NIEHS Other: E. W. Van Stee Head, Inhalation Toxicology Group EBCB NIEHS		
COOPERATING UNITS (if any) Duke University Inhalation Toxicology Section, Environmental Biology and Chemistry Branch, NIEHS		
LAB/BRANCH Laboratory of Behavioral and Neurological Toxicology		
SECTION Behavioral Toxicology Group		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.45	PROFESSIONAL: 0.15	OTHER: 0.30
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this study is to assess changes in neurobehavioral functioning of rats at various times during and after exposure to carbon disulfide by inhalation. Changes in the functioning of <u>catecholaminergic receptors</u> will also be determined in animals dosed with carbon disulfide.		

PROJECT DESCRIPTIONS

METHODS EMPLOYED: Male, albino rats of the Fischer strain are exposed to 2 mg/l of carbon disulfide (CS_2) by inhalation 4 hours per day, 5 days per week for 6 consecutive weeks. At weeks 3 and 6 during dosing and at 1 and 3 weeks after cessation of dosing, rats are assessed for neurobehavioral functioning using the following measures: exploratory motor activity, startle responsiveness, rectal temperature, performance on an inclined screen, forelimb grip strength, hind limb extensor response, visual placement responses, and gross behavioral observations of sensory/motor functioning. Body weights are taken weekly during the course of the experiment.

Some rats will be tested after 6 weeks of dosing and 3 weeks after cessation of dosing for changes in the sensitivity of catecholaminergic functioning. Spontaneous motor activity, startle responsiveness, and stereotypic motor responses following systemic administration of d-amphetamine and/or apomorphine will be assessed.

MAJOR FINDINGS AND PROPOSED COURSE: Depending upon the findings in the experiment described above, higher or lower doses of CS_2 and different exposure times may be studied.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies will aid in the elucidation of the mechanism of action of CS_2 , as well as provide information as to appropriate tests to be used in the study of changes in brain functioning during repeated exposure to neurotoxins.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90005-01 LBNT
PERIOD COVERED January 1, 1978 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Effects of Equal Doses of Firemaster FF-1 and 2,4,5,2',4',5'-Hexabromobiphenyl in Rats		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: H. A. Tilson Head, Behavioral Toxicology Group LBNT NIEHS Other: P. A. Cabe Senior Staff Fellow LBNT NIEHS		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Behavioral and Neurological Toxicology SECTION Behavioral Toxicology Group		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.75	PROFESSIONAL: 0.25	OTHER: 0.50
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The neurobehavioral effects of 2,4,5,2',4',5'-hexabromobiphenyl (HBB) and Firemaster FF-1, a commercial mixture of polybrominated biphenyls (PBB), of which HBB is the major component, have been shown to be different when given in amounts equating HBB content. The present study repeats and extends observations of neurobehavioral effects of equal doses of HBB or FF-1. Rats are trained to press a lever in standard operant conditioning chambers to avoid an unsignalled presentation of electric footshock and to escape shock when it is presented (unsignaled continuous avoidance) until a stable baseline of responding is achieved. Dosing (10 mg/kg of FF-1, 10 mg/kg of HBB, or corn oil vehicle) is then started. Effects on performance of the avoidance schedule are to be evaluated. Parallel assessment on a screening test battery is to include the following tests: <u>body weight</u> , <u>sensorimotor reflexes</u> and <u>orientation</u> , <u>hind limb strength</u> , <u>forelimb strength</u> , <u>visual placement</u> , <u>inclined screen</u> , <u>spontaneous activity</u> , <u>startle</u> , and <u>rectal temperature</u> . Latency to escape shock in a second apparatus will be tested. Autonomic signs and general health indicators will be noted.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Three groups of rats were initially trained in standard operant conditioning chambers to press a lever in daily (5/week) sessions to avoid electric shock or to terminate it (escape) when it occurred. When responding stabilized, dosing by gavage with 10 mg/kg Firemaster, 10 mg/kg HBB, or corn oil vehicle was started. Concurrent evaluations on a primary screening test battery are made. Such measures include: spontaneous activity, sensorimotor reflexes and orientation (tail tip curl reflex; foot drop; olfactory, visual, and auditory localization; corneal reflex; pinna reflex; tail and toe pinch responses; tilt compensation reflex; and lateral hop), forelimb grip strength, and startle. Rectal temperature and body weights are taken, and autonomic signs and general health indicators are noted. Latency to escape and avoid signalled foot shock is also measured.

MAJOR FINDINGS AND PROPOSED COURSE: This study is in progress.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Interactive effects of chemical substances, especially in commercial-grade preparations, will be a continuing problem in environmental health. PBB toxicity is of immediate concern because it has affected relatively large human populations. Evaluation of substances such as PBB, which clearly affect human health, is specifically within the mandate of the Institute.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90006-01 LBNT
PERIOD COVERED March 1, 1978 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Neurobehavioral Toxicity of Developmental Exposure to 2,4-Diphenoxylacetic Acid (2,4-D) in Japanese Quail		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: H. A. Tilson Head, Behavioral Toxicology Group LBNT NIEHS		
COOPERATING UNITS (if any) Zoology Department, North Carolina State University Poultry Science Department, North Carolina State University		
LAB/BRANCH Laboratory of Behavioral and Neurological Toxicology		
SECTION Behavioral Toxicology Group		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.30	PROFESSIONAL: 0.10	OTHER: 0.20
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) In spite of their wide use, the <u>behavioral and neurological toxicity of herbicides</u> , such as <u>2,4-diphenoxylacetic acid (2,4-D)</u> , have not been well studied. The purpose of the present study is to investigate the effects of exposing Japanese quail (<u>Coturnix coturnix japonica</u>) eggs to varying concentrations of commercially available and technical grade 2,4-D on the <u>development of neurobehavioral functioning</u> .		

PROJECT DESCRIPTION

METHODS EMPLOYED: Fertilized Japanese quail eggs will be exposed to varying concentrations of 2,4-D, Weedar-64 and a vehicle of inert substances. After incubation, the effects of hatching time, percentage hatched, and growth rates of quail will be assessed. At 6 to 8 weeks of age, the mating behavior and reproductive capabilities of male and female quail of each group will be assessed. At six months of age and every six months thereafter, spontaneous motor activity, flight thrust, and startle responsiveness to a visual stimulus will be evaluated. Acquisition and retention of a shuttle box avoidance response will also be assessed. Body weights will be recorded weekly.

At 6 to 8 weeks of age, other groups of quail treated in the egg with 2,4-D will be food-deprived to 80-85% of their free-feeding body weight and trained to peck a key for food reinforcement. After baseline performance has been established, the quail will be trained to respond on a FR FI schedule of food reinforcement. Acquisition of operant behavior and baseline performance parameters will be evaluated for indications of neurotoxicity.

MAJOR FINDINGS AND PROPOSED COURSE: This project will soon be underway. The results from this study will be used to determine the usefulness of the quail as a subject for study of herbicides, as well as provide the basis for future studies with other agents.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Herbicides such as 2,4-D are often regarded as relatively safe environmental agents because of the manner of application in relation to the time and method of crop harvest and the rapid biodegradability of many herbicides. The effects of exposure to herbicides and their metabolites on developing organisms are relatively unknown and are in need of systematic scientific evaluation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90007-01 LBNT								
PERIOD COVERED April 1, 1978 to September 30, 1978										
TITLE OF PROJECT (80 characters or less) Neurobehavioral Toxicity of Neonatal Exposure of Rats to Diethylstilbestrol										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">H. A. Tilson</td> <td style="width: 35%;">Head, Behavioral Toxicology Group</td> <td style="width: 15%;">LBNT NIEHS</td> </tr> <tr> <td>Other:</td> <td>C. Lamartiniere</td> <td>Senior Staff Fellow</td> <td>LET NIEHS</td> </tr> </table>			PI:	H. A. Tilson	Head, Behavioral Toxicology Group	LBNT NIEHS	Other:	C. Lamartiniere	Senior Staff Fellow	LET NIEHS
PI:	H. A. Tilson	Head, Behavioral Toxicology Group	LBNT NIEHS							
Other:	C. Lamartiniere	Senior Staff Fellow	LET NIEHS							
COOPERATING UNITS (if any) Laboratory of Environmental Toxicology, NIEHS										
LAB/BRANCH Laboratory of Behavioral and Neurological Toxicology										
SECTION Behavioral Toxicology Workgroup										
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709										
TOTAL MANYEARS: 0.85	PROFESSIONAL: 0.35	OTHER: 0.50								
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS										
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this project is to determine the effects of neonatal exposure to <u>diethylstilbestrol</u> and <u>testosterone</u> on the <u>neurobehavioral</u> functioning of rats at various times during development. A battery of simple, diagnostic tests designed to assess a variety of <u>behavioral</u> and <u>neurological</u> functions will be used.										

PROJECT DESCRIPTION

METHODS EMPLOYED: Sprague-Dawley rats will be given propylene glycol, testosterone propionate or diethylstilbestrol subcutaneously postnatally. The offspring will be allowed to stay with their natural mother or cross-fostered until they are weaned at 21 days of age. Equal numbers of males and females having different mothers will be selected for testing.

At 21 and 61 days of age, the rats will be assessed in a routine behavioral and neurological examination which will include the following measures: spontaneous motor activity (exploratory behavior), forelimb grip strength, hind limb extensor responding, performance on the inclined screen, visual placement, startle responsiveness, learning and retention of a one-way avoidance response, rectal temperature, and body weight. A battery of gross observational tests concerning responsiveness to pain, visual, auditory, and olfactory stimuli, as well as responses to change in body position, will also be employed.

MAJOR FINDINGS AND PROPOSED COURSE: Assessment of animals exposed neonatally to diethylstilbestrol and testosterone is currently underway. Results from these studies will be employed in planning further work using different doses and injection times.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: There is a need to understand more fully the effects of perinatal exposure to diethylstilbestrol and other hormonal agents on neurobehavioral functions other than reproductive and sexual behaviors. The ability to detect other, more subtle behavioral teratogenic effects may prove useful in determining the presence of previously unknown or poorly described psychological and neurobehavioral deficits. In addition, such studies may prove useful in elucidating the mechanism and site of action of such agents.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90008-01 LBNT						
PERIOD COVERED November 1, 1977 to September 30, 1978								
TITLE OF PROJECT (80 characters or less) Acute and Chronic Neurobehavioral Toxicity of Acrylamide in Rats								
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%;"> <tr> <td style="width: 33%;">PI: H. A. Tilson</td> <td style="width: 33%;">Head, Behavioral Toxicology Group</td> <td style="width: 33%;">LBNT NIEHS</td> </tr> <tr> <td>Other: P. A. Cabe</td> <td>Senior Staff Fellow</td> <td>LBNT NIEHS</td> </tr> </table>			PI: H. A. Tilson	Head, Behavioral Toxicology Group	LBNT NIEHS	Other: P. A. Cabe	Senior Staff Fellow	LBNT NIEHS
PI: H. A. Tilson	Head, Behavioral Toxicology Group	LBNT NIEHS						
Other: P. A. Cabe	Senior Staff Fellow	LBNT NIEHS						
COOPERATING UNITS (if any) Albert Einstein College of Medicine of Yeshiva University, Bronx, New York								
LAB/BRANCH Laboratory of Behavioral and Neurological Toxicology								
SECTION Behavioral Toxicology Group								
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709								
TOTAL MANYEARS: 0.80	PROFESSIONAL: 0.40	OTHER: 0.40						
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (a1) MINORS ☐ (a2) INTERVIEWS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER								
SUMMARY OF WORK (200 words or less - underline keywords) <u>Acrylamide</u> is a known neurotoxin having effects generally most easily seen as <u>peripheral neuropathy</u>. In order to define the validity of a primary screening test battery more clearly, rats were studied under acute and chronic dosing. A lethal dose study (orally, by gavage) showed an LD50 of 251 mg/kg at 24 hours and 175 mg/kg at 168 hours. Dosing at 50-200 mg/kg showed <u>hind limb motor dysfunction</u> (hind limb extensor response, inclined screen) at 12 hours post-dosing. Hind limb recovery was complete at 168 hours. <u>Forelimb strength</u> was not affected. Repeated dosing (10-20 mg/kg by gavage) produced hind limb dysfunction at cumulative doses of 50-100 mg/kg over 4 weeks; doses up to 400 mg/kg did not produce forelimb dysfunction. Partial recovery was seen after cessation dosing. Dosing over 90 days is being assessed in a more extended battery including measures of: <u>body weight</u>, <u>sensorimotor reflexes</u> and <u>orientation</u>, <u>spontaneous activity</u>, <u>forelimb grip strength</u>, <u>hind limb strength</u>, <u>visual placement</u>, <u>inclined screen coordination</u>, <u>startle</u>, and <u>rectal temperature</u>. <u>Autonomic signs</u> and general health indicators are also noted. <u>Morphological assessment</u> of medulla oblongata and sciatic nerve will also be made.								

PROJECT DESCRIPTION

METHODS EMPLOYED: Subjects have been Fischer strain albino rats, typically 70 days of age at the beginning of an experiment. They are housed individually with food and water freely available.

Measures taken have included the following: spontaneous motor activity (automated activity cages), forelimb grip strength, visual placement, inclined screen coordination, hind limb strength, and startle. Rectal temperature is taken and observations of autonomic signs and general health indicators (including body weight) are made. Animals are tested before dosing (by gavage) in the first week of dosing and at 3 week intervals thereafter, where repeated doses are given. At the end of the dosing period and at 1 month after cessation of dosing, selected animals will be sacrificed and sections of medulla oblongata and sciatic nerve inspected for signs of acrylamide toxicity.

MAJOR FINDINGS AND PROPOSED COURSE:

- (1) Lethal dose. A lethal dose (LD50) of 251 mg/kg was found at 12 hours and 175 mg/kg at 168 hours.
- (2) Acute dosing. Caudal motor dysfunction was observed in the hind limb extensor response and inclined screen tests 12 hours after a single dose of 50-200 mg/kg. Hind limb function recovered completely by 168 hours. No effect on forelimb strength was observed.
- (3) Subacute dosing. Repeated 10-20 mg/kg doses over four weeks produced hind limb deficits at cumulative doses of 50-100 mg/kg. No forelimb dysfunction appeared. Recovery was incomplete at 2 weeks post-dosing.
- (4) Chronic dosing. Repeated 5-20 mg/kg doses (3/week) over a 120 day period is in progress. The extended battery of tests listed above is administered every third week. Preliminary analyses suggest effects on caudal motor function but no obvious effects on sensory function. The experiment will terminate in April 1978; neuropathological analyses on peripheral nerves will be performed on contract.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The validation of neurobehavioral test methods is a crucial step in the development of neurotoxicological predictive capabilities. The studies described here provide an invaluable data base for further methods development, using a substance which in itself is a known environmental contaminant. The studies described are congruent with the Institute's mission to discover methods for the detection and prediction of environmental health hazards.

PUBLICATIONS

Cabe, P. A., Tilson, H. A., Mitchell, C. L., and Dennis, R.: A simple recording grip strength device. Pharmac. Biochem. Behav. 8: 101-102, 1978.

Tilson, H. A. and Cabe, P. A.: A strategy for the assessment of neurobehavioral consequences of environmental factors. Environ. Health Perspec. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90009-01 LBNT												
PERIOD COVERED March 1, 1978 to September 30, 1978														
TITLE OF PROJECT (80 characters or less) Neurobehavioral Toxicity of Developmental Exposure of Japanese Quail to Microwave Irradiation														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">H. A. Tilson</td> <td style="width: 35%;">Head, Behavioral Toxicology Group</td> <td style="width: 15%;">LBNT NIEHS</td> </tr> <tr> <td>Other:</td> <td>D. I. McRee</td> <td>Research Physicist</td> <td>LEB NIEHS</td> </tr> <tr> <td></td> <td>C. L. Mitchell</td> <td>Laboratory Chief</td> <td>LBNT NIEHS</td> </tr> </table>			PI:	H. A. Tilson	Head, Behavioral Toxicology Group	LBNT NIEHS	Other:	D. I. McRee	Research Physicist	LEB NIEHS		C. L. Mitchell	Laboratory Chief	LBNT NIEHS
PI:	H. A. Tilson	Head, Behavioral Toxicology Group	LBNT NIEHS											
Other:	D. I. McRee	Research Physicist	LEB NIEHS											
	C. L. Mitchell	Laboratory Chief	LBNT NIEHS											
COOPERATING UNITS (if any) Laboratory of Environmental Biophysics, NIEHS Zoology Department, North Carolina State University Poultry Science Department, North Carolina State University														
LAB/BRANCH Laboratory of Behavioral and Neurological Toxicology														
SECTION Behavioral Toxicology Group														
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709														
TOTAL MANYEARS: 0.30	PROFESSIONAL: 0.10	OTHER: 0.20												
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords) The long-term <u>neurobehavioral</u> effects of perinatal exposure of Japanese Quail eggs (<u>Coturnix coturnix japonica</u>) to 2450 MHz CW <u>microwave irradiation</u> at a power density of 5 mW/cm² for the first 12 days of development will be assessed in this experiment. Measures include <u>sexual and reproductive behaviors</u>, a battery of <u>behavioral and neurological measurements</u>, <u>learning and retention</u>, and <u>baseline performance on an operant schedule</u>.														

PROJECT DESCRIPTION

METHODS EMPLOYED: Fertilized Japanese quail eggs will be exposed to 2450 MHz CW microwave irradiation at a power density of 5 mW/cm² for the first 12 days of development. The effects of irradiation on hatchability and number of deformities of exposed and control eggs will be observed.

At 6 to 8 weeks, the mating behavior and reproductive capabilities of 10 male and 10 female quail from each group will be assessed. Measures of neurobehavioral functioning (startle responsiveness, flight thrust, spontaneous motor activity) will be obtained at six months of age and every six months thereafter for a period of up to 2 years. The ability to acquire a two-way active avoidance response will be tested at 6 months of age and retention testing will occur every 6 months. General health indicators (body weight, food consumption) will also be assessed.

A second group of 10 exposed and 10 control male quail will be food-deprived to 80-85% of their free-feeding body weight at 6-8 weeks of age and trained to peck a lighted key for food reinforcement. Following measures of acquisition abilities, the quail will be trained to respond on a mult FR FI schedule. Baseline parameters regarding responding under discriminative control and patterning of responding under various schedule components will be assessed.

MAJOR FINDINGS AND PROPOSED COURSE: This project will soon be underway. Results from this study will be used to determine the usefulness of the quail as a subject for behavioral teratological research and for the study of physical factors such as irradiation. This experiment will also be the basis for future investigations concerning the effects of microwave irradiation on neurobehavioral development.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Microwave-generating devices are employed in a variety of military, industrial, and civilian applications. However, the consequences of prenatal exposure to microwave irradiation on neurobehavioral functioning have not been studied systematically, in spite of the known sensitivity of developing organisms to many types of chemical and physical insults.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90010-01 LBNT												
PERIOD COVERED October 1, 1977 to September 30, 1978														
TITLE OF PROJECT (80 characters or less) Neurobehavioral Effects of Chronic Polybrominated Biphenyls in Rats and Mice														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">PI:</td> <td style="width: 30%;">H. A. Tilson</td> <td style="width: 30%;">Head, Behavioral Toxicology Group</td> <td style="width: 10%;">LBNT NIEHS</td> </tr> <tr> <td>Other:</td> <td>P. A. Cabe</td> <td>Senior Staff Fellow</td> <td>LBNT NIEHS</td> </tr> <tr> <td></td> <td>J. A. Moore</td> <td>Associate Director</td> <td>RRP NIEHS</td> </tr> </table>			PI:	H. A. Tilson	Head, Behavioral Toxicology Group	LBNT NIEHS	Other:	P. A. Cabe	Senior Staff Fellow	LBNT NIEHS		J. A. Moore	Associate Director	RRP NIEHS
PI:	H. A. Tilson	Head, Behavioral Toxicology Group	LBNT NIEHS											
Other:	P. A. Cabe	Senior Staff Fellow	LBNT NIEHS											
	J. A. Moore	Associate Director	RRP NIEHS											
COOPERATING UNITS (if any) Research Resources Program, NIEHS Duke University														
LAB/BRANCH Laboratory of Behavioral and Neurological Toxicology														
SECTION Behavioral Toxicology Group														
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709														
TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.40	OTHER: 0.60												
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords) <u>Polybrominated biphenyls (PBB)</u> have been reported to produce clinical signs of <u>neurological toxicity</u> in humans and neurobehavioral deficits in <u>rats</u> and <u>mice</u>, both in relatively short exposures. The present work is intended to assess neuro-behavioral consequences of chronic (six month) exposure to a commercial mixture of PBB (Firemaster FF-1). Rats receive 0.3, 1.0, or 3.0 mg/kg body weight of FF-1 or corn oil vehicle. Mice receive 1.0, 3.0, or 10.0 mg/kg body weight of FF-1, or vehicle. Both male and female <u>rats</u> and <u>mice</u> are to be tested in a battery designed to screen for neurobehavioral dysfunction. Tests for rats include: <u>body weight</u>, <u>motor activity</u>, <u>sensorimotor reflexes</u> or orientation, <u>visual placement</u>, <u>forelimb grip strength</u>, <u>inclined screen</u>, <u>hind limb strength</u>, <u>startle to air puff</u>, and <u>rectal temperature</u>. <u>Shock escape</u> latencies and acquisition of <u>shock avoidance</u> are tested at the end of one month's dosing and retention tested at 2 and 6 months. Mice receive the same tests except for hind limb strength, inclined screen, startle, and lateral hop. Autonomic/general health observations and food consumption measures are taken on all animals.														

PROJECT DESCRIPTION

METHODS EMPLOYED: Rats and mice are dosed orally on a 5 day/week schedule for 180 days, at 0.3, 1.0, or 3.0 mg/kg (rats) or 1.0, 3.0, 10.0 mg/kg (mice). Controls of both species are dosed with corn oil vehicle.

Rats are administered the following tests: spontaneous motor activity (automated monitors), sensorimotor reflexes and orientation (tail tip curl reflex; foot drop; orientation to visual, auditory, and olfactory stimuli; corneal reflex; pinna reflex; response to toe and tail pinch; tilt compensation reflex; lateral hop), forelimb grip strength, visual placement, inclined screen, hind limb extensor response, and startle. Latency to avoid signalled shock and acquisition of shock avoidance are tested. Retention tests later in the study are scheduled. Mice are tested in a limited version of the battery, excepting the inclined screen, hind limb strength, and startle tests. Autonomic signs and general health indicators (including rectal temperature, food consumption, and body weight) are also noted for all animals.

At the end of the dosing period, some subjects will be tested in a neurophysiological battery consisting of measures of nerve conduction speed, maximal firing rates, and duration of maximal rates. Attempts to measure the strength of the hind limb reflex will also be made.

MAJOR FINDINGS AND PROPOSED COURSE: This study is currently in progress.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Chronic effects of environmental toxins are of continuing concern in environmental medicine. The controlled evaluation of effects of PBB is a current problem in human health, in that an affected population and a population at risk exist; the Institute's programs are aimed specifically at dealing with such environmental health issues.

LABORATORY OF BIOCHEMICAL GENETICS

LABORATORY OF BIOCHEMICAL GENETICS Summary Statement

The primary objective of the program in the Laboratory of Biochemical Genetics is focused on development of systems to monitor the human population for induction of mutations in somatic and germinal cells and to evaluate the risk of exposure of the human population to environmental pollutants. The Laboratory of Biochemical Genetics was organized in the Spring 1978. The accomplishments of the goals of the Laboratory of Biochemical Genetics are vital for the general health of the human population. In order to reach the goals within the shortest possible time, several mechanisms are used. These include:

1. Development of an intramural research program based on in-house research by staff scientists and support of work at other institutions in the collaborative program with staff scientists as project officers.

2. Development of better mechanisms for information exchange. These include: (a) support of the Environmental Mutagen Information Center (EMIC) and (b) serving as associate editor or members of the editorial board of journals which publish research in the area of environmental mutagenesis.

3. Development of better mechanisms for training young scientists for research careers in environmental mutagenesis at: (a) the predoctoral level and (b) the postdoctoral level.

INTRAMURAL RESEARCH PROGRAM

The intramural staff has been organized into two programs. The major research accomplishments in each program are as follows:

In the Mutation Monitoring Group, the mechanism for induction of mutations in mammalian cells in vivo and in vitro are being sought. Drs. Mallin and Ansari are utilizing the various hemoglobins of the inbred strains of mice which differ in as many as thirteen amino acids. An immunological comparison has been done of these hemoglobins. The immunological differences among them were found to correlate with amino acid sequence differences. It is essential for the mutation monitoring systems to isolate monospecific antibodies. Until now no technique has existed by which the presence of monospecific antibodies could be easily demonstrated. This technique has now been developed. Mutations can be detected either by using single cells or by using the radioimmunoassay to measure the presence of the mutant hemoglobin in red blood cell lysates.

The specificity of the radioimmunoassay is being tested by using radioactive hemoglobin synthesized in vitro.

Another mutation system is being developed which is based on use of mono-specific antibodies to lactic-dehydrogenase-X. Dr. Malling and Mr. Burkhardt now have isolated monospecific antibodies to mouse lactic-dehydrogenase-X. This antibody does not react with rat sperm. It, therefore, should be possible to detect mutations in rat sperm where an amino acid normally present in rat has been exchanged with one normally present in mice. Previously, we had developed histochemical stains for succinyl dehydrogenase and α -glycerophosphate dehydrogenase. We are presently surveying the mouse strains for variation in thermoability of these two enzymes in situ in the sperm.

In the biochemical specific locus mutation system, offspring of animals treated with procarbazine are presently being screened for presence of mutations. Drs. Johnson, Roberts, and Sharma are developing a long series of additional tests on the micro centrifugal fast analyzer which can be done simultaneously with the electrophoretic analysis.

COLLABORATIVE RESEARCH PROGRAM

In the Collaborative Research Program most of the contracts fall naturally within three areas: (1) development of systems for monitoring of the human population for mutations, (2) development of risk extrapolation systems to predict induced mutation rate in the human population, and (3) dissemination of mutagenesis information. The contracts will be described following this outline.

1. Mutation monitoring systems for the human population

In a contract at the University of Washington, an attempt is being made to develop a simple system to measure point mutations in readily accessible human somatic cells. This assay is based on changes in the expected frequency of human red blood cells containing mutant hemoglobin rather than normal hemoglobin. Specific antisera are being developed to detect red blood cells, each of which carry one of ten different hemoglobins. By conjugating the antiserum with different colored fluorescent dye, mutant RBC's will fluoresce.

2. Risk extrapolation systems

a. Biochemical mutations. A contract with The Jackson Laboratory involves the incorporation of 13 additional mouse enzyme alleles into the C57BL/6J strain. This will directly complement the electrophoretic assay system developed at our Institute and will significantly enhance the sensitivity of the system to detect biochemical specific locus mutations.

In a contract with the Research Triangle Institute, the progeny from treatment of the male parent with procarbazine is presently being screened.

b. Polygenic mutations. A contract with the Georgia Institute of Technology attempts to develop a polygenic assay for the detection of point mutations in mice. By studying changes in the means and variances of characters determined by many genes, it should be possible to detect genetic changes which do not express major phenotypic effects.

3. Collection and dissemination of mutagenesis literature

During the past year we have continued to support EMIC (Environmental Mutagen Information Center) by interagency agreement at the Oak Ridge National Laboratory. This center has over 22,000 bibliographic entries in its data banks and contains as a unique world-wide resource for information in the area of environmental chemical mutagenesis.

INFORMATION EXCHANGE

Journals - Staff members serve as associate editors, assistant editors, or members of editorial boards of Mutation Research, Environmental Health Perspectives, The Journal of Toxicology, and Reviews in Genetic Toxicology. This makes it possible not only to promote publication of papers dealing with various research areas of environmental mutagenesis but also to promote development of new methods for publication.

TRAINING PROGRAMS

The great shortage of scientists to do research in the area of environmental mutagenesis both in the United States and abroad has provided incentive for staff scientists to develop a training program at both the predoctoral and postdoctoral levels. At the postdoctoral level, staff scientists include three visiting fellows from two foreign countries.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60019-05 LBG										
PERIOD COVERED October 1, 1977 through September 30, 1978												
TITLE OF PROJECT (80 characters or less) The Use of Male Germinal Tissue in the Detection of Mutational Events												
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">H. V. Malling</td> <td style="width: 20%;">Laboratory Chief</td> <td style="width: 10%;">LBG</td> <td style="width: 20%;">NIEHS</td> </tr> <tr> <td>Other:</td> <td>J. G. Burkhardt</td> <td>Chemist</td> <td>LBG</td> <td>NIEHS</td> </tr> </table>			PI:	H. V. Malling	Laboratory Chief	LBG	NIEHS	Other:	J. G. Burkhardt	Chemist	LBG	NIEHS
PI:	H. V. Malling	Laboratory Chief	LBG	NIEHS								
Other:	J. G. Burkhardt	Chemist	LBG	NIEHS								
COOPERATING UNITS (if any) Environmental Biology & Chemistry Branch Biometry Branch												
LAB/BRANCH Laboratory of Biochemical Genetics SECTION Mutation Monitoring Group												
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, NC 27709												
TOTAL MANYEARS: 0.7	PROFESSIONAL: 0.3	OTHER: 0.4										
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS												
SUMMARY OF WORK (200 words or less - underline <u> The aim of this project is to develop systems for measuring the frequency of point mutations and DNA change in male germinal tissue. These systems would have the advantage of testing the human population by means of readily available samples, i.e., spermatozoa. At present enzyme histochemical methods are being developed to detect point mutations in <u>male mice</u> . The histochemical staining technique has been quantatized for α -glycerophosphate dehydrogenase and succinyl dehydrogenase. Preliminary work has started with the Axiomat scanning microscope to determine its usefulness in measuring the distribution of DNA content in a population of sperm. It is hoped that this instrument may prove useful in showing changes in the distribution pattern in sperm populations derived from mice treated or untreated with known mutagens.												

PROJECT DESCRIPTION

METHODS EMPLOYED: Some enzymes in sperm can be detected readily by histochemical methods. The enzymes under study in this laboratory are α -glycerophosphate dehydrogenase and succinate dehydrogenase.

The principle of these studies is that, with the use of standardized staining procedures and use of thermodenaturation, it should be feasible to detect thermoresistant mutations in the spermatozoal enzymes. These mutations will be estimated by counting stained and unstained cells with the aid of a Zeiss Axiomat microscope connected to a PDP-12 computer and a teleprinter output. Information will also be collected on magnetic tape for processing in the Institute's PDP-11 computer.

MAJOR FINDINGS AND PROPOSED COURSE: At present, it has been possible to standardize the staining technique to the extent that it is reproducible after repeated experimentation. The characteristics of the thermodenaturation of α -glycerolphosphate dehydrogenase and succinyl dehydrogenase has been established in situ. Similar studies will be carried out with the enzymes in vitro. Ten to twenty different mouse strains will be screened for natural variation in the thermosensitivity of these two enzymes.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Development of this system is important, because it would permit the detection of mutational events within a single individual that could be used to monitor the human population. Besides, it would also enable us to detect the genetic consequences of exposure to mutagens.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65001-01 LBG										
PERIOD COVERED October 1, 1977 through September 30, 1978												
TITLE OF PROJECT (80 characters or less) Immunological Comparison of Mouse Hemoglobins												
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">A. A. Ansari</td> <td style="width: 35%;">Visiting Associate</td> <td style="width: 10%;">LBG</td> <td style="width: 5%;">NIEHS</td> </tr> <tr> <td>Other:</td> <td>H. V. Malling</td> <td>Laboratory Chief</td> <td>LBG</td> <td>NIEHS</td> </tr> </table>			PI:	A. A. Ansari	Visiting Associate	LBG	NIEHS	Other:	H. V. Malling	Laboratory Chief	LBG	NIEHS
PI:	A. A. Ansari	Visiting Associate	LBG	NIEHS								
Other:	H. V. Malling	Laboratory Chief	LBG	NIEHS								
COOPERATING UNITS (if any) None												
LAB/BRANCH Laboratory of Biochemical Genetics												
SECTION Mutation Monitoring Group												
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, NC 27709												
TOTAL MANYEARS: 0.7	PROFESSIONAL: 0.4	OTHER: 0.3										
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS												
SUMMARY OF WORK (200 words or less - underline) Mouse hemoglobins are being used in this laboratory to develop immunological methods for the study of mutation in mammals <u>in vivo</u> . An <u>immunological comparison</u> of these hemoglobins was, therefore, essential. Different hemoglobin variants were compared in radioimmunoassay and the immunological differences among them were found to correlate with amino acid sequence differences.												

PROJECT DESCRIPTION

METHODS EMPLOYED: A solid phase radioimmunoassay, utilizing ^{125}I labeled hemoglobin and Sepharose-bound anti-hemoglobin antibody, was used to compare the immunological properties of different mouse hemoglobins.

MAJOR FINDINGS AND PROPOSED COURSE: Immunological properties of several mice hemoglobins bearing α -chains 1 or 4, and β -chains, d_{maj} or p_{min} , were compared using radioimmunoassays that involved inhibition by these hemoglobins of a reaction between ^{125}I labeled C57BL/6 hemoglobin (α chain 1, β chain s) and horse antibody against C57BL/6 hemoglobin. SJL hemoglobin (α chains 1 and 4, β chain s) and C57BL/6 hemoglobin were found to have identical relative association constants, $K_{O(rel)}$. The d_{maj} hemoglobins from DBA/2 and AU/Ss mice (α chain 1, β chain d_{maj}) were 3.3 to 3.6 times less reactive while the p_{min} hemoglobin from AU/Ss mice was found to be 17 times weaker than C57BL/6 hemoglobin. These immunological differences correlate with the amino acid sequence differences in the β chains.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND PROGRAM OF THE INSTITUTE: One of our major goals is to study mutation utilizing mouse hemoglobin and monospecific antibodies. Immunological comparison of different mouse hemoglobins is necessary to interpret data obtained from such mutation studies.

PUBLICATIONS

Ansari, A. A., Bahuguna, L. M., Jenison, M., and Malling, H. V. (1978) Immunological comparison of mouse hemoglobins. Immunochemistry, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65002-01 LBG										
PERIOD COVERED October 1, 1977 to September 30, 1978												
TITLE OF PROJECT (80 characters or less) Mutation Studies using Hemoglobin and Monospecific Antibodies against Hemoglobins												
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">A. A. Ansari</td> <td style="width: 35%;">Visiting Associate</td> <td style="width: 10%;">LBG</td> <td style="width: 5%;">NIEHS</td> </tr> <tr> <td>Other:</td> <td>H. V. Malling</td> <td>Laboratory Chief</td> <td>LBG</td> <td>NIEHS</td> </tr> </table>			PI:	A. A. Ansari	Visiting Associate	LBG	NIEHS	Other:	H. V. Malling	Laboratory Chief	LBG	NIEHS
PI:	A. A. Ansari	Visiting Associate	LBG	NIEHS								
Other:	H. V. Malling	Laboratory Chief	LBG	NIEHS								
COOPERATING UNITS (if any) None												
LAB/BRANCH Laboratory of Biochemical Genetics												
SECTION Mutation Monitoring Group												
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, NC 27709												
TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.6	OTHER: 0.3										
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS												
SUMMARY OF WORK (200 words or less - underline) This project is a part of our goal to study <u>mutation in mammals in vivo</u> utilizing <u>monospecific antibodies against hemoglobin variants</u> . Antisera against two mouse Hb's have been raised in horses and rabbits. A system has been developed that allows detection of the monospecific antibodies. Conditions are being optimized for labeling of <u>red blood cells</u> with the FITC-labeled antibodies. A <u>sandwich technique</u> for the labeling was developed for these cells. Monospecific antibodies are being purified for use in the system.												

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: Several antisera against DBA/2 (D2) and C57BL/6 (C57) hemoglobin (Hb) have been raised in horses and rabbits. Different pools of the antisera were found to contain 0.2 to 2.2 mg precipitating antibody/ml of serum when titrated with homologous (immunizing) Hb. Sharp precipitin bands were observed with homologous as well as heterologous hemoglobins in gel diffusion. Purification of monospecific antibody from these sera is in progress. This involves absorbing the serum with the heterologous Hb-immunoabsorbent to remove the cross reacting antibodies; the absorbed serum is then passed through a homologous Hb immunoabsorbent column. The bound antibody is eluted with an acidic pH buffer. This last step results in the elution of significant amounts of Hb along with the antibody. Experiments are being carried out to find an efficient way of removing the contaminating Hb without losing too much antibody.

Red blood cells from D2 and C57 mice could be successfully labeled with FITC-conjugated purified anti-Hb antibodies. However, this technique required FITC labeling of each antibody preparation which results in significant loss of the purified antibody. A 'sandwich' immunofluorescent technique has been developed that utilizes FITC-labeled goat anti-horse IgG. This technique precludes the need for FITC-labeling of the anti Hb antibody and is also more sensitive than the direct method.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND PROGRAM OF THE INSTITUTE: These studies are directed toward developing a methodology for studying mutation in mammals in vivo utilizing immunological technique. Different hemoglobin variants have been compared for their immunological cross reactivity and antisera against them have been raised. Staining of the cells on slides has been worked out. Monospecific antibodies are being purified for use in the mutation detection system. Using this system, chemicals and pollutants could be screened for their mutagenic activity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65003-01 LBG
PERIOD COVERED October 1, 1977 through September 30, 1978		
TITLE OF PROJECT (80 characters or less) Development of a Double Antibody Radioimmunoassay for Hemoglobin		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: A. A. Ansari Other: H. V. Malling	Visiting Associate Laboratory Chief	LBG NIEHS LBG NIEHS
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Biochemical Genetics		
SECTION Mutation Monitoring Group		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, NC 27709		
TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.2	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline key words) High sensitivity of <u>radioimmunoassays</u> (RIA) allows detection of nanogram and picogram quantities of protein molecules. Theoretically, therefore, RIA could also be used for the detection of the small amounts of mutant molecules. This project was undertaken to develop a double antibody RIA for <u>hemoglobins</u> and to explore the possibility of using the RIA for the detection of <u>mutant hemoglobin</u> molecules.		

PROJECT DESCRIPTION

METHODS EMPLOYED: The procedure for the radioimmunoassay is as follows. One hundred microliters of an appropriately diluted horse anti-mouse hemoglobin antiserum are mixed with 100 μ l of iodinated mouse hemoglobin in Falcon tubes. The reaction mixtures are placed on a rotary drum in the cold room for 24 h. This is followed by the addition of 100 μ l of goat anti-horse IgG antiserum which has been previously diluted so as to give maximum precipitation of the complexes. Within 30 min of this, 200 μ l of 125 g/liter polyethylene glycol is added, and after 1 h in the cold, the tubes are centrifuged. The supernatants are discarded by aspiration and the residues are washed two times with 3 ml of PBS before counting in the γ -counter. The percentage of bound radioactivity is calculated as a function of total radioactivity.

MAJOR FINDINGS AND PROPOSED COURSE: The first phase of the RIA, i.e., the initial reaction between the antigen and the antibody, was found to be complete within 24 h. The reaction proceeded better at 4°C than at 25°C. The second phase, i.e., separation of bound from unbound antigen, was achieved by precipitation with a second antibody (goat anti-horse IgG) and polyethylene glycol (PEG). A 50 g/liter concentration of PEG was found to be best suited for the assay. Mixing of all the reagents together was found to decrease the binding as compared to the system in which second antibody and PEG were added after completion of the first phase. Maximum precipitation of the complex took place within 30 min after the addition of the second antibody and 1 h after the addition of 50 g/liter PEG.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND PROGRAM OF THE INSTITUTE: The RIA system described here combines the conventional double antibody RIA with the PEG method. This method has decreased the amount of time necessary for precipitation from 24 h (or longer) to 1 h. Large molecular weight antigens could not be estimated in the conventional PEG method because of their insolubility in 200 g/liter PEG utilized in the assay. The use of a low concentration of PEG along with the second antibody in the method described here allows the estimation of large molecular weight antigens. This double antibody-PEG method has a general applicability for small as well as large molecular weight antigens.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65004-01 LBG
PERIOD COVERED October 1, 1977 through September 30, 1978		
TITLE OF PROJECT (80 characters or less) Immunological Characterization of Hypoxanthine-Guanine Phosphoribosyl Transferase from Mouse Liver		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Other:	L. M. Bahuguna A. A. Ansari H. V. Malling	Visiting Fellow Visiting Associate Laboratory Chief
		LBG LBG LBG
		NIEHS NIEHS NIEHS
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Biochemical Genetics		
SECTION Mutation Monitoring Group		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, NC 27709		
TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.8	OTHER: 0.1
CHECK APPROPRIATE BOX(ES)		
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER		
☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline key words) The purpose of this study is to detect HGPRT variants in different inbred strains of mouse. The enzyme has been purified from mouse liver. <u>Antibodies</u> are being raised in rabbits.		

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: The enzyme HGPRT was purified from mouse liver. Its homogeneity was checked by electrophoretic methods. Antisera are being raised in rabbits. The antibody will be used to detect HGPRT variants in different strains of mice.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND PROGRAM OF THE INSTITUTE: Monospecific antibodies against the HGPRT variants would provide a powerful tool for studying mutation in the mammalian cells in vivo. Antiserum against one HGPRT variant could be absorbed with HGPRT of another strain and the monospecific antibody thus obtained could be used for studying mutation at the HGPRT locus by the immunofluorescence technique being developed in this laboratory

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65005-01 LBG
PERIOD COVERED October 1, 1977 through September 30, 1978		
TITLE OF PROJECT (80 characters or less) Detection of Monospecific Antibody against Hemoglobin		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Other:	A. A. Ansari H. V. Malling	Visiting Associate Laboratory Chief
		LBG LBG
		NIEHS NIEHS
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Biochemical Genetics		
SECTION Mutation Monitoring Group		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, NC 27709		
TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.3	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline k- Monospecific antibodies against hemoglobin variants can be used for the detection of <u>mutation</u> at the Hb loci. No method exists for the detection of these monospecific antibodies. We are developing a simple and rapid procedure for the detection of monospecific antibodies against hemoglobin variants.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Immunoabsorbents prepared by coupling hemoglobin with CNBr-activated Sepharose are used.

MAJOR FINDINGS AND PROPOSED COURSE: Horse antisera immunized with C57BL/6 hemoglobin (Hb) were allowed to react with C57BL/6Hb and DBA/2 Hb immunoabsorbents. After thorough washing, goat anti-horse IgG coupled with fluorescein isothiocyanate was added to each of the reacted immunoabsorbents. Strong fluorescence in both the immunoabsorbents was recorded. However, non-immune horse sera also gave strong fluorescence in the test. Experiments are planned to solve this problem and to develop this staining procedure for the detection of monospecific antibodies.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND PROGRAM OF THE INSTITUTE: Purification of monospecific antibodies against hemoglobins is a lengthy procedure. No method exists to detect the presence of monospecific antibodies. In absence of such a detection method, sometimes all the lengthy procedures of purification are carried out just to find out at the end that the serum did not contain any monospecific antibody.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65006-01 LBG										
PERIOD COVERED October 1, 1977 through September 30, 1978												
TITLE OF PROJECT (80 characters or less) Comparison of Double Antibody and Solid-Phase Radioimmunoassays												
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI:</td> <td style="width: 33%;">A. A. Ansari</td> <td style="width: 33%;">Visiting Associate</td> <td style="width: 10%;">LBG</td> <td style="width: 10%;">NIEHS</td> </tr> <tr> <td>Other:</td> <td>H. V. Malling</td> <td>Laboratory Chief</td> <td>LBG</td> <td>NIEHS</td> </tr> </table>			PI:	A. A. Ansari	Visiting Associate	LBG	NIEHS	Other:	H. V. Malling	Laboratory Chief	LBG	NIEHS
PI:	A. A. Ansari	Visiting Associate	LBG	NIEHS								
Other:	H. V. Malling	Laboratory Chief	LBG	NIEHS								
COOPERATING UNITS (if any) None												
LAB/BRANCH Laboratory of Biochemical Genetics												
SECTION Mutation Monitoring Group												
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, NC 27709												
TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.3	OTHER: 0.3										
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS												
SUMMARY OF WORK (200 words or less - underline key words) We are developing <u>radioimmunoassays</u> (RIA) for the study of mutation in hemo- globins. This project was undertaken to compare two RIA procedures, namely, <u>double antibody RIA</u> and <u>solid phase RIA</u> . The former method was found to be more sensitive than the solid phase RIA.												

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: Two commonly used radioimmunoassay (RIA) procedures, double antibody assay and solid phase assay, have been compared using the same reagents and conditions. Mouse hemoglobin-horse anti-mouse hemoglobin system was chosen for this study. The antibody was found to have more binding capacity in soluble form than in the Sepharose-bound form. The inhibition curves in both the assay systems were shifted to higher inhibitor concentration at higher control binding levels. At 40% control binding level, the double antibody RIA was found to be 2.7 times more sensitive than the solid phase RIA. The reason for this most probably lies in the lower binding capacity of the Sepharose-bound antibody which necessitates the use of higher amounts of the antibody to reach 40% control binding.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND PROGRAM OF THE INSTITUTE: Determination of mutant proteins requires sensitive methods capable of detecting trace amounts of the mutant molecules. Comparison of different methods, as done here, allows selection of the best detection method for a particular system.

PUBLICATIONS

Ansari, A. A., Bahuguna, L. M., and Mallig, H. V. (1978) Comparison of double and solid phase radioimmunoassays. J. Immunol. Methods, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65007-01 LBG
PERIOD COVERED October 1, 1977 through September 30, 1978		
TITLE OF PROJECT (80 characters or less) Study of Mutation by Using Sperm Specific Enzyme LDH-X		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Other:	A. A. Ansari H. V. Malling J. G. Burkhart	Visiting Associate Laboratory Chief Chemist
		LBG LBG LBG
		NIEHS NIEHS NIEHS
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Biochemical Genetics		
SECTION Mutation Monitoring Group		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, NC 27709		
TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.5	OTHER: 0.4
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline) This project is designed to study mutagenesis using monospecific antibodies against the <u>sperm specific enzyme, lactic dehydrogenase-X</u> . The antiserum against mouse LDH-X has been characterized and its cross reaction with rat LDH-X has been studied. An immunofluorescent technique has been developed to label the mouse and rat sperm using the horse anti-LDH-X antibody and FITC-labeled goat anti-horse IgG antibody.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Gel-diffusion tests were carried out in 1.5% agar gels. Antibody concentration was determined by precipitin titration.

MAJOR FINDINGS AND PROPOSED COURSE: A pool of the horse antiserum against mouse LDH-X was found to have 4 to 5 mg of antibody per milliliter of the serum. It gave single precipitin line with mouse LDH-X in gel diffusion test. The antiserum showed a strong cross reaction with rat LDH-X giving a "spur" in the gel diffusion test. When precipitin analysis was done, 2-3 mg/ml of the antibody, accounting for approximately half of the anti-mouse LDH-X antibody, could be precipitated by rat LDH-X. The portion of the anti-LDH-X antibody that could not be precipitated by rat LDH-X could contain some monospecific antibody that would react with mouse LDH-X but not with rat LDH-X. Attempts are being made to purify this monospecific antibody. Animals will also be immunized with rat LDH-X to allow purification of a second monospecific antibody that would react with rat LDH-X but not with mouse LDH-X.

Labeling of the sperm - A sandwich technique has been developed to label the sperm with immunofluorescence. The sperm is allowed to react with the horse anti-mouse LDH-X serum followed by reaction with FITC labeled goat anti-horse IgG. Both mouse and rat sperm give strong fluorescence with the horse anti-mouse LDH-X serum. However, normal horse serum also confers similar fluorescence to the rat and mouse sperm. Experiments are being carried out to investigate and eliminate this nonspecific fluorescence. In this context, the FITC labeled anti-horse IgG was purified by DEAE-cellulose chromatography, and the purified fraction having F.P ratio of 1 was found to be specific. Tissue fluorescence has also been eliminated by using filters in the microscope.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies are a part of the program to study mutation in mammals in vivo. The immunodiffusion and precipitin titration experiments have pointed to the possibility of the presence of monospecific antibodies against LDH-X in the serum. These antibodies will now be purified. The immunofluorescence technique has demonstrated that the sperm can be labeled with these antibodies.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60021-06 LBG															
PERIOD COVERED October 1, 1977 through September 30, 1978																	
TITLE OF PROJECT (80 characters or less) Gene Mutation Indicators in Mice																	
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">F. M. Johnson</td> <td style="width: 35%;">Research Geneticist</td> <td style="width: 10%;">LBG</td> <td style="width: 5%;">NIEHS</td> </tr> <tr> <td>Other:</td> <td>G. Roberts</td> <td>Visiting Fellow</td> <td>LBG</td> <td>NIEHS</td> </tr> <tr> <td></td> <td>R. Sharma</td> <td>Visiting Fellow</td> <td>LBG</td> <td>NIEHS</td> </tr> </table>			PI:	F. M. Johnson	Research Geneticist	LBG	NIEHS	Other:	G. Roberts	Visiting Fellow	LBG	NIEHS		R. Sharma	Visiting Fellow	LBG	NIEHS
PI:	F. M. Johnson	Research Geneticist	LBG	NIEHS													
Other:	G. Roberts	Visiting Fellow	LBG	NIEHS													
	R. Sharma	Visiting Fellow	LBG	NIEHS													
COOPERATING UNITS (if any) Research Triangle Institute, Divisions of Biology and Analytical Sciences, Research Triangle Park, NC; North Carolina State University, Departments of Statistics and Animal Science, Raleigh, NC																	
LAB/BRANCH Laboratory of Biochemical Genetics																	
SECTION Physiological Genetics Group																	
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, NC 27709																	
TOTAL MANYEARS: 5.0	PROFESSIONAL: 3.0	OTHER: 2.0															
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">☐ (a) HUMAN SUBJECTS</td> <td style="width: 33%;">☐ (b) HUMAN TISSUES</td> <td style="width: 33%;">☒ (c) NEITHER</td> </tr> <tr> <td colspan="3">☐ (a1) MINORS ☐ (a2) INTERVIEWS</td> </tr> </table>			☐ (a) HUMAN SUBJECTS	☐ (b) HUMAN TISSUES	☒ (c) NEITHER	☐ (a1) MINORS ☐ (a2) INTERVIEWS											
☐ (a) HUMAN SUBJECTS	☐ (b) HUMAN TISSUES	☒ (c) NEITHER															
☐ (a1) MINORS ☐ (a2) INTERVIEWS																	
SUMMARY OF WORK (200 words or less - underline) Electrophoretic methods and spectrophotometrically determined <u>enzyme</u> <u>characteristics</u> are being investigated for their value to indicate newly induced <u>mutational events</u> in <u>mice</u> .																	

PROJECT DESCRIPTION

The objective of this work is the investigation and evaluation of various possible technical approaches for their potential and suitability to detect the presence of newly induced gene (point) mutations in mice and by inference, other mammals and humans. In progress is work with starch gel electrophoresis (which extends and replaces two previously reported projects (Z01-ES-60054-01 LEM and Z01-ES-60021-05 LEM) and enzyme characteristics determined spectrophotometrically. As this is a new project, no major findings have resulted to date.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: No practical methodology exists for the determination of newly induced point mutations in mammals and such mutations may, individually and/or in accumulation, be associated with effects detrimental to the health and well being of present and future human populations. As work on the project continues, additional analytical approaches appropriate to the detection and quantitation of a variety of molecular species will be investigated in concert with the application of conventional mutation test methodologies.

THE JACKSON LABORATORY - BAR HARBOR, MAINE
(NIH-N01-ES-5-2159)

TITLE: Development of a New Mouse Strain to Maximize the Sensitivity of a Point Mutation Assay

CONTRACTOR'S PROJECT DIRECTOR: Thomas H. Roderick, Ph.D.

PROJECT OFFICER (NIEHS): F. M. Johnson, Ph.D.

DATE CONTRACT INITIATED: February 19, 1974

CURRENT ANNUAL LEVEL: \$42,000

PROJECT DESCRIPTION

Strains of mice having different alleles homozygous at common loci between them offer potential for the detection of newly induced point mutations. For example, two strains having one such locus, with one of the strains treated with an experimental mutagen and with individual of the two strains mated after treatment,

aa x AA (treated)

provide the opportunity to detect among the normally expected offspring,

Aa

the exceptional individuals of the genotype,

aa

caused by the mutation,

A → a

in the gametes of the treated individual

If there are several of such differences between two strains and if the characteristics are attributable to identifiable gene products, such as specific enzymes, then a system capable of detecting a host of particular mutational events becomes available. The advantage of having heterozygous individuals for the normally expected class of individuals is that when certain enzyme characteristics are utilized, the properties of both parents may be expected to be present in the normal F₁ offspring. But with the occurrence of a mutation at the controlling locus, one or the other of the parental characteristics may be missing or altered in the F₁, depending on the nature of the mutation and in which parent mutation took place.

This contract is for the construction of a special strain of mice which differs to the maximum practical extent for alleles at many loci from the standard DBA/2J strain. Variant alleles not found in either DBA/2J or C57BL/6J are introduced into the C57BL/6J background genotype by mating and repeated backcrossing, the latter being conducted with appropriate monitoring to assure the continued presence of existing allelic differences between the two strains.

Electrophoretically expressed enzyme characteristics are presently being utilized. A series of matings and backcrosses have been conducted, but a strain with any significant advantage over C57BL/6J is yet to be developed.

There is no practical system presently available with which it is possible to detect systematically and efficiently newly induced point mutations and to study their biological effects in mammals. This contract serves the purpose of developing such a capability.

INFORMATION CENTER COMPLEX/INFORMATION DIVISION
OAK RIDGE NATIONAL LABORATORY - OAK RIDGE, TENNESSEE 37830
(Interagency Agreement Between NIEHS and ORNL)

TITLE: Environmental Mutagen Information Center

CONTRACTOR'S PROJECT DIRECTOR: Mr. John S. Wassom

PROJECT OFFICER (NIEHS): H. V. Malling, Ph.D.

COLLABORATING INSTITUTES: National Cancer Institute; Energy Research and
Development Administration

DATE CONTRACT INITIATED: July 1, 1970

CURRENT ANNUAL LEVEL: \$200,000

PROJECT DESCRIPTION

OBJECTIVES: The mission of the Environmental Mutagen Information Center (EMIC) is to collect, organize, and disseminate chemical mutagenesis information.

MAJOR FINDINGS AND PROPOSED COURSE: EMIC's data file at the end of March 1978 contained 22,100 bibliographic entries. Of these, approximately 21,000 have been indexed with respect to agent(s), organism(s), and Chemical Abstract Service Registry Number (s). The Center processes requests for information at a rate of 2-3 per day. These requests come primarily from governmental agencies in the United States or government supported research in universities. Several requests from other countries are also received. The EMIC data bank can now be screened by Toxline. EMIC will continue to monitor the world scientific literature for reports on chemical mutagenesis. It will issue its annual literature survey in the middle of the fiscal year. This report will include an index to test organisms or test objectives as well as indexes for agents and selected title keywords. As great a portion as possible of EMIC's total bibliographic entries will be keyworded with respect to agent, test organism, and test object. EMIC will continue to answer selected requests for special information for the scientific, educational, and industrial communities. Tabular extracts of the data from literature will be added to the data file as they are prepared in conjunction with the timely state-of-the-art reports on subjects of current scientific interests.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many compounds with widespread distribution in man's environment are known to be mutagenic in laboratory test organisms. Man's constant contact with mutagens may pose more danger to the genetic health of the human population than does radiation. The awareness of this problem among scientists, administrators, and laymen created a need to make reference to published reports on tests for chemical mutagenesis readily available. This is important because these reports are scattered throughout a great number of publication sources (1550).

EMIC has helped several national commissions in collecting and extracting the data from the literature. For the Institute, and especially the Laboratory of Biochemical Genetics, EMIC saves more than one man-year of time for the personnel in the Laboratory by supplying their information needs.

PUBLICATIONS

Authors: Wassom, J. S., Shelby, M. D., Von Halle, E. S., Beauchamp, R. O., Jr., Owens, E. T., Miller, I. R., Barnard, W. J., and Adams, J. O. Chemical Mutagenesis: A survey of the 1975/1976 literature. Oak Ridge National Laboratory Publication No. ORNL/EMIC-10. Oak Ridge National Laboratory, Oak Ridge, TN, 1978, 502 pp.

Authors: Wassom, J. S., Shelby, M. D., and Von Halle, E. S.: Mutagenesis and Teratogenesis Information Programs. In, Proceedings of the Symposium on the "Handling of Toxicology Information," Bethesda, MD. (in press)

Authors: Wassom, J. S. and Malling, H. V.: Specialized Information in Toxicology: I. Environmental Mutagen Information Center. In, Advances in Modern Toxicology, eds., W. G. Flamm and M. A. Mehlman, 1978.

Brown, M. L., Wassom, J. S., Malling, H. V., Shelby, M. D., and Von Halle, E. S.: Non-mammalian assay systems used in the evaluation of selected chemical compounds for mutagenic activity / A literature summary. J. Nat. Cancer Inst., in press.

Wassom, J. S., Huff, J. E., and Loprieno, N.: A review of the genetic toxicology of chlorinated dibenzo-p-dioxins. Mutation Research, in press.

GEORGIA INSTITUTE OF TECHNOLOGY - ATLANTA, GA 30332
(NIH-N01-ES-5-2135)

TITLE: Development of a Polygenic Assay for Point Mutations in Mice

CONTRACTOR'S PROJECT DIRECTOR: John W. Crenshaw, Jr., Ph.D.

PROJECT OFFICER (NIEHS): F. M. Johnson, Ph.D., Research Geneticist

DATE CONTRACT INITIATED: June 26, 1975

CURRENT ANNUAL LEVEL: \$76,500

PROJECT DESCRIPTION

The purpose of this contract is to investigate mutagenic effects in mice of some known mutagens, as measured by mean and variance changes in polygenic characters. The contractor is investigating the effects of mutagen-induced polygenic mutations on a series of traits in F₁ and F₂ generation progenies descended from mutagenized males of inbred strains of mice. Progeny are examined for traits such as age of eruption of incisors, body weight, sex ratio, righting response, and hematocrit. Different strains and mutagens will be studied. Results have been mixed in experiments conducted to date. Characteristics subject to change as a result of mutation at any of a number loci may be useful to the detection of mutagenic activity associated with environmental substances. This approach is in contrast with other work which attempts to detect mutagenic change in characters controlled by individual specific genes.

UNIVERSITY OF WASHINGTON - SEATTLE, WASHINGTON
(NIH-NIEHS-74-2151)

TITLE: Detection of Point Mutations in Somatic Cells

CONTRACTOR'S PROJECT DIRECTOR: G. Stamatoyannopoulos, Ph.D.

PROJECT OFFICER (NIEHS): Heinrich V. Malling, Ph.D.

DATE CONTRACT INITIATED: January 1, 1974

CURRENT ANNUAL LEVEL: \$167,000

PROJECT DESCRIPTION

OBJECTIVES: It is likely that an increase in the mutation rate in the human population would be detrimental, yet there is no system developed to monitor the human population for point mutations. The purpose of this investigation is to develop a simple system to measure point mutations in readily accessible human somatic cells. It is likely that the somatic cell mutation rate would reflect the germ cell mutation rate, and the rate of introduction of genetic defects into the human population.

METHODS EMPLOYED: Many different types of hemoglobin mutations exist in the human population. Some of these are point mutations in the α - and β -chain. Since such mutations occur in the germinal tissue, it is reasonable to assume that they also occur in the stem cells for the red blood cells (RBC). This type of mutation should result in an RBC which would contain an aberrant hemoglobin. By using a monospecific antibody to a number of aberrant hemoglobin types, such cells should be detected in samples from normal individuals after reaction of the antibodies with various fluorescent dyes. Antibodies are being produced against HbS, HbC, HbOArab, HbE, HbQ India, Hb Ottawa, Hb Hasharon, Hb Cranston, Hb Wayne, and Hb Constant Spring.

MAJOR FINDINGS AND PROPOSED COURSE: Fluorescent antibodies to the α -chain frameshift mutant hemoglobin Wayne has been produced. The purification was difficult because of interference from Hb A₂ which apparently cross reacts with Hb Wayne. Artificial mixtures of various proportions of blood from Hb Wayne patients and normal showed the expected ratio of Hb Wayne cells when screened with anti-Wayne FICT. The intensity of the Wayne cells were much less than the Sickle cells after reaction with fluorescence monospecific antibodies.

In order to supply Lawrence Livermore Laboratory with monospecific fluorescent antibodies, the production of anti-Hb S has continued. The new technique developed at Lawrence Livermore Laboratory for staining of red blood cells in suspension has been tested with anti-Hb S FICT, and the results were very encouraging.

Antibodies to an expanding list of hemoglobin variants will be produced and used to investigate somatic cell mutation rates with respect to age and to investigate populations at mutagenic risk such as patients receiving cancer chemotherapy and those exposed to radiation. Screening programs would be able to identify populations exposed to mutagenic substances.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A system able to detect mutations in the genome of readily accessible somatic cells would allow estimation of the mutation rate in the human population. It would allow investigation of individuals and populations exposed to a mutagenic risk. There is no simple technique for screening at present. The antisera would also be useful in investigation of thalassemia and related genetic hemoglobin disorders.

PUBLICATIONS

Papayannopoulou, Th., Lim, G., McGuire, T. C., Ahern, V., Nute, P. E., and Stamatoyannopoulos, G.: Use of specific fluorescent antibody for the identification of hemoglobin C in erythrocytes. Am. J. Hematol. 2:105-112, 1977.

Papayannopoulou, Th., Nute, P. E., Stamatoyannopoulos, G., and McGuire, T. C.: Hemoglobin ontogenesis: Test of the gene excision hypothesis. Science 197: 1215-1216, 1977.

Stamatoyannopoulos, George: Possibilities for demonstrating point mutations in somatic cells as illustrated by studies of mutant hemoglobins. Experts Conference on Genetic Damage Caused by Environmental Agents, Oslo, Norway, May 11-13, 1977, in press.

Papayannopoulou, Th., Rosse, W., and Stamatoyannopoulos, G.: Fetal hemoglobin in paroxysmal nocturnal hemoglobinuria. Evidence for random derivation of the Hb F containing erythrocytes (F cells) from the PNH clone and from normal hemopoietic stem cell lines. Blood, in press.

TITLE: Cytophysical Studies in Mutagenesis

CONTRACTOR'S PROJECT DIRECTOR: Mortimer L. Mendelsohn, Ph.D.

PROJECT OFFICER (NIEHS): Heinrich V. Malling, Ph.D.

COLLABORATING INSTITUTES: Department of Energy

DATE CONTRACT INITIATED: September 23, 1977

CURRENT ANNUAL LEVEL: \$317,000

PROJECT DESCRIPTION

OBJECTIVES: Development of system for monitoring of the human population for mutations in vivo in single cells depends on the detection of a rare event in a single cell which is most effectively done by using flow systems. The scope of this contract is to develop such systems for red blood cells and sperm.

MAJOR FINDINGS AND PROPOSED COURSE: (1) The detection of somatic mutations in erythrocytes. At the present time it takes about one man-month to manually estimate the spontaneous mutation rate from Hb A to Hb S in a human. The counting, therefore, is a bottleneck for the future of this work, both for the screening of the human population and for the experimental backup work in mammals. Part of the effort in this contract is to develop a technique for staining red blood cells with monospecific antibodies in suspension for later sorting in the flow systems.

(2) Cytochemical fluorochromatic methods for dehydrogenase. It is possible to base a lot of mutation testing systems in somatic cell and in sperm in vivo on detection of variant dehydrogenases. At the present time we use nitro bluetetrazolium to detect the dehydrogenases and, to find the variant cell we use a scanning microscope. This procedure, however, is very slow; and it would be a great step forward if we could use a cell sorter to sort the variant cells from the normal cells. All cell sorters are based, however, on detection of fluorescent signals. It is, therefore, important to develop dehydrogenase stains which can fluoresce. Part of the effort in this contract is to attach a fluorescent molecule to the tetrazolium molecule so a fluorescent dehydrogenase stain can be developed.

(3) Distribution of DNA content in mice with various translocations. Detection of transmissible translocations is a very important tool for establishing risk of chemicals for the human population. At the present time the detection of translocations in mice after treatment of the parent requires a complex breeding program. During the meiosis the mouse, heterozygotic for translocation, will produce sperm with varying amounts of DNA. A question we ask here is, "Is that varying amount of DNA sufficient that we can detect a

mouse, which is heterozygotic for translocation among normal ones, by comparing the variance of DNA in the sperm between the two mice?" The measurement of the DNA in sperm can be done on the flow system. A sufficient number of sperm can very easily be measured, and the variance can be compared. Since the mouse sperm is flat, there are severe problems in doing that. By rearranging the optical path in the flow system, we hope to overcome these difficulties.

SIGNIFICANCE TO THE BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Many compounds with widespread distribution in man's environment are known to be mutagenic or carcinogenic in laboratory test organisms. Man's constant contact with mutagens may pose more danger to the genetic health of the human population than does radiation. Nevertheless we do not have available today a monitoring system for the human population. Through collaboration of our in-house research, University of Washington, and this contract, we hope to develop such systems.

LABORATORY OF ENVIRONMENTAL BIOPHYSICS

LABORATORY OF ENVIRONMENTAL BIOPHYSICS Summary Statement

The research program of the Laboratory of Environmental Biophysics is concerned with two main areas: the biological effects of physical factors present in our environment and the molecular interactions that occur between environmental agents and their biological targets (Molecular Biophysics). The physical factors under current investigation include non-ionizing radiation (microwaves) and noise (including both auditory and non-auditory) effects. The Molecular Biophysics Program is mainly focussed on the use of sophisticated spectroscopic techniques to monitor the interaction of environmental agents with nucleic acids, membranes, proteins and microsomal systems.

Non-ionizing Radiation

The objectives of the non-ionizing radiation research program are as follows: 1) to develop microwave exposure systems for bioeffects research, 2) to develop and test techniques for measuring microwave energy absorption, 3) to determine the effect of microwaves on isolated nerve preparations, 4) to determine how 2450 MHz microwave radiation interacts with biological systems at all levels, 5) to study the effect of long-term exposure of experimental animals to 915 MHz and 2450 MHz microwave radiation on their CNS and behavior, 6) to ascertain the effect of 60 Hz fields from high voltage transmission lines on the CNS of mammals.

During the past year further refinements have been made on a waveguide system for exposing isolated nerve preparations to microwave radiation. These changes now permit the maintenance of both the control and exposed nerves at a constant temperature. In addition the survival time of isolated nerves has been extended by the use of mineral oil in the neural stimulating and recording electrode modules. With the aid of the new exposure system it has been possible to demonstrate changes in the excitability and refractoriness in nerves exposed to continuous wave 2450 MHz microwave radiation.

Research into the biological effects of microwaves at the cellular and organ level has continued. The number of chromatid exchanges in the bone marrow of mice was not affected by exposure of the animals to 2450 MHz CW microwave radiation (20 mW/cm²) for 8 hrs/day for 28 days. A study on the effects of 2450 MHz CW radiation on rat lymphocytes in culture also failed to uncover any significant differences between exposed and control cells. Despite the report that the heart rate of perfused rat hearts is affected by microwave exposure no significant change in the heart rate of embryonic quail hearts exposed to 2450 MHz CW radiation was detected. These studies will be repeated with pulsed microwave radiation of different intensities. However, a significant reduction in sperm mobility was observed in male quail that had been

exposed to microwave radiation in ovo. A decreased percentage of fertile eggs was also seen when these exposed males were mated with either exposed or non-exposed females.

One of the most important areas of concern in non-ionizing radiation research is the effect of long term, low level exposure. The long term, low level effects of microwave exposure to 915 (918) MHz and 2450 MHz radiation is currently the subject of investigation by two contractors. A significant behavioral deficit during acquisition of a conditioned avoidance was observed in adult rats that had been exposed to 918 MHz CW radiation in utero. All of these tests are now being repeated after exposure to 2450 MHz CW radiation. Changes in the level of blood electrolytes were seen in female rats exposed to 2450 MHz at 500 $\mu\text{W}/\text{cm}^2$ for 7 hours/day, 7 days a week for 3 months. These same animals also exhibited altered behavioral responses at the end of 3 months exposure. A decrease in serum total free sulfhydryl groups was also observed. Finally, groups of rats exposed to 60 Hz electric fields have shown changes in body weight, urine output and adrenal weight. No differences were observed either in thyroid weight or in hematological parameters.

Noise

At present, approximately two-thirds of the noise program research effort is directed toward elucidating either basic mechanisms of noise induced hearing loss, providing better techniques for physiological assessment of such losses or providing the means for accurately reproducing specific noise exposure environments. The rest of the effort is focused on reproductive, teratogenic, or cardiovascular effects, though a major effort toward determining the parameters associated with possible fetal hearing loss in guinea pigs resulting from maternal exposure has been initiated.

Areas under current investigation include (1) the role of electrolyte composition and flux on the functioning of the guinea pig cochlea, (2) the development of an optical fiber motion detector for auditory system measurements, (3) the development of electrophysiological techniques to recover speech from the cochleas of live, anesthetized guinea pigs, (4) the effect of noise and ototoxic agents, e.g., methyl mercury on the electrolyte metabolism of the cochlea, (5) the effect of ototoxic insult on the coding of complex signals in the auditory system and (6) the development of an impact noise generator for hearing loss studies, (7) the possible fetal hearing loss occurring when female guinea pigs are exposed to occupational exposure levels.

One of the major goals of the noise program is to understand the basic mechanisms of electrolyte transport in the guinea pig cochlea under normal conditions and under the influence of noise and chemical agents. Exposure of guinea pigs to white band noise for 7 days (16 hours on, 8 hours off) produced a number of changes including a suppression of the brainstem evoked response to clicks, a suppression of the cochlear microphonics and a variable decrease in the rate of uptake of ^{43}K into the endolymph. Perfusion of the perilymphatic space with ethacrynic acid resulted in a decrease in the sound evoked response and the endocochlear potential. A decrease in ^-Cl uptake into the endolymph was also observed. When overstimulated with broadband white noise the cochlear microphonics and action potential were decreased while the endolymph and perilymph potassium concentrations were unchanged.

Work has continued on the development of an optical fiber motion detector for measurement of the ossicular chain at displacement levels (1-100 Angstrom) corresponding to normal levels (40-80 dB SPL). An analysis has been completed for the more complex case of illumination with non-collimated light source. Results indicate that very high radiance tungsten lamps may be a viable alternative to the laser for illumination purposes.

Development of an improved closed system electroacoustic transducer for noise exposure studies has also begun. Preliminary work suggests that these devices may be superior to conventional loudspeaker systems.

A review of impact noise, its mechanism, control and effects has been completed. A prototype impact noise generator has been built and tested. The sound pressure levels produced by this model reached 140 dB which is well within the range necessary to simulate industrial exposures of 120-160 dB peak. A final model, now in the production state, will be tested for suitability in laboratory exposure studies.

Experiments to test the effect of prenatal exposure to high noise levels on subsequent auditory thresholds have been initiated. In these studies, pregnant guinea pigs are being exposed to weaving room noise in an acoustically shielded chamber 8 hours/day, 5 days/week. When exposure has been completed both the sound evoked brainstem response, N_1 potential and the cochlear microphonics of the offspring will be measured.

Finally the effect of protracted noise exposure on cardiovascular function is being examined via a contract. In these experiment rhesus monkeys are being exposed for periods of up to one year to noise conditions resembling those found in the community and workplace. The cardiovascular response of these exposed animals will be compared with that of a control group maintained in relative quiet for the same time period.

Molecular Biophysics

The main objective of the Molecular Biophysics Program is to understand at the molecular level the interaction of environmental agents with target biological systems including nucleic acids, proteins membranes and microsomal system. For these studies a number of high sophisticated spectroscopic techniques, e.g., electron spin resonance and nuclear magnetic resonance spectroscopy, fluorescence and absorption spectroscopy, circular dichroism and stopped flow spectrometry are being employed.

Nucleic acids. It is now widely recognized that many mutagenic agents exert their biological effect by modifying DNA. The purpose of this project is to synthesize base pair specific probes which can be used to monitor the interaction of mutagens with nucleic acids. The acridine dyes, which bind strongly to DNA, are known to be mutagenic. Spin labeled analogs of 9-aminoacridine, containing the stable nitroxide free radical, have therefore been synthesized and their binding to nucleic acids studied by means of a number of techniques, e.g., electron spin resonance, fluorescence spectroscopy and sedimentation. Results with these labels indicate, however, that while they do intercalate

into DNA they do not exhibit base pair specificity. Nevertheless these compounds do appear to be useful probes for monitoring the interaction of DNA with histones. In contrast to the acridines, actinomycin-D and adriamycin are known to be specific for G-C and A-T pairs respectively. Spin-labeled analogs of these two agents are therefore being synthesized and will be tested for base pair specific binding. If these spin labels retain the specificity of the parent molecules then they will be used to monitor the interaction of known mutagens, including the polycyclic hydrocarbons, with nucleic acids.

Membranes. A large number of chemical agents are known to cause mast cell degranulation with the concomitant release of histamine. While the initial event must be the binding of these agents to the cell membrane little is known about the subsequent molecular events that ultimately lead to degranulation. We have therefore studied the interaction of compound 48/80 (a classical mast cell degranulating agent) with a number of membrane systems in an attempt to elucidate the molecular mechanism of histamine release. Because of the difficulty obtaining purified mast cells in large quantities much of the initial work has been done with a mouse mast cell tumor line and with human red cell ghost preparations. Initial results, employing both fluorescent and spin probe techniques, have shown that 48/80 binds strongly to the surface of cell membranes. Fluorescence microscopy has indicated that the binding is uniform and that there is no patching or capping. No evidence could be found for a 48/80 induced change in the fluidity of membranes. One striking observation was an increase in the light scattering properties of both cells and isolated red cell ghost membranes upon exposure to 48/80. This phenomenon may be due to aggregation of cell membrane proteins since similar changes are also seen after heat treatment. A new procedure has now been developed for the purification of larger quantities of rat peritoneal mast cells. This will greatly facilitate our studies since it is now possible to carry out most of the experiments on the known biological target cells.

Proteins. A comprehensive study of the interaction of heavy metal ions with proteins has been started. Initial work will be concerned with the binding of copper to human serum albumin (HSA). This protein was selected for study because of its central role in the transportation of metal ions in the blood stream. Previously it has been shown that the N-terminal portion of HSA is involved in the binding of copper. We are in the process of synthesizing the N-terminal tripeptide of HSA for spectroscopic studies of its interaction with copper. These studies will be extended to peptides prepared from HSA by enzymatic digestion and finally to HSA itself. Other metal ions including cobalt, nickel, cadmium and manganese will also be examined. It is hoped that this approach will provide useful information on the nature of metal ion binding sites in proteins.

Microsomal systems. It is now generally accepted that the toxicity of many xenobiotics results from the generation of highly reactive species (free radicals, singlet oxygen, superoxide anion) during metabolism. A new program has therefore been started to develop techniques which will permit the identification of such reactive intermediates during the metabolism of various chemical agents by microsomal systems. Initial work will focus on the generation of nitroaromatic anion free radicals from a nitrofuran carcinogen and from 4-nitroquinoline-1-oxide. Attempts will be made, using electron spin

resonance spectroscopy, to detect these free radicals and to follow their fate in various biological systems. Subsequent studies will also involve a search for free radical metabolites of polycyclic aromatic hydrocarbons. Attempts will also be made to detect the superoxide anion directly in a microsomal system by means of electron spin resonance spectroscopy.

Personnel

Several major personnel changes occurred during the year. Dr. C.F. Chignell assumed the responsibility of Chief. Dr. R.P. Mason (Research Chemist), Dr. B.K. Sinha (Senior Staff Fellow), Dr. M.J. Ortner (Staff Fellow), Mr. R.H. Sik (Biologist) and Mr. H.G. Davis (Electronics Technician) also joined the Laboratory. Other additions to the Laboratory were: Visiting Fellows: Dr. P. Mohanakrishnan (Dr. Chignell) and Dr. B. Kalyanaraman (Dr. Mason); Intergovernmental Personnel Act, Dr. George Read (Dr. Chignell).

Other Activities

Dr. Colin F. Chignell: member of the NIH ad hoc Neuropharmacology Study Section; NIEHS representative, US-USSR Cooperative Program on Photobiology; NIEHS representative, International Workshop on Solar Radiation Damage and the Induction of Skin Cancer, Editorial Board member: Molecular Pharmacology, Medicine, Environmental Health Perspectives. Invited speaker at the NIGMS Pharmacology-Toxicology Symposium, Washington, D.C., November 17-18, 1978; invited speaker at the Membrane Receptors and Disease Conference, Bethesda, Maryland, March 23-24, 1978.

Dr. Reginald O. Cook: member, Interagency Noise Effects Panel; member, Committee of Hearing, Bioacoustics and Biomechanics of the National Research Council, National Academy of Science; member, Working Group 83 on Noise Effects on the Fetus; invited lecturer, seminars on effects of noise - courses on speech, hearing and acoustical engineering, UNC and NCSU.

Dr. Teruzo Konishi: member of Technical Committee, Physiological and Psychological Section, Acoustical Society of America.

Dr. Ronald P. Mason: Invited speaker at the Gordon Conference on Drug Metabolism, August 1978.

Dr. Donald I. McRee: Adjunct appointment, NCSU; Coordinator, US-USSR Cooperative Program on Health Effects of Non-ionizing Radiation; NIEHS representative on Inter-departmental Radiation Advisory Committee (IRAC) on Biological Effects of Non-ionizing Radiation; Chairman of Scientific Information Exchange Working Group; representative for DHEW on Interagency Advisory Committee on Electric Field Effects from High Voltage Transmission Lines (organized by ERDA); Chairman of ad hoc Working Group of the Office of Science and Technology Policy (OSTP) to review the state of knowledge and research needs concerning biological effects of nonionizing radiation; representative of American-National Standards Institute C-95 Committee on Safety Standards of Nonionizing Radiation.

Dr. Mary J. Ortner: member of Organization of Intramural Scientists, member of Animal Experimentation Committee.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50005-05																									
PERIOD COVERED October 1, 1977 to September 30, 1978																											
TITLE OF PROJECT (80 characters or less) Effects of Noise and Ototoxic Agents on Energy Balance and Metabolism in Cochlea																											
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																											
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%; vertical-align: top;">PI:</td> <td style="width: 35%;">Teruzo Konishi</td> <td style="width: 30%;">Visiting Scientist</td> <td style="width: 10%;">LEB</td> <td style="width: 10%;">NIEHS</td> </tr> <tr> <td style="vertical-align: top;">Other:</td> <td>Alec Salt</td> <td>Visiting Fellow</td> <td>LEB</td> <td>NIEHS</td> </tr> <tr> <td></td> <td>Philip E. Hamrick</td> <td>Senior Scientist</td> <td>RRP</td> <td>NIEHS</td> </tr> <tr> <td></td> <td>Edward Lebetkin</td> <td>Biological Technician</td> <td>LEB</td> <td>NIEHS</td> </tr> <tr> <td></td> <td>Charles Hamm</td> <td>Physical Science Tech.</td> <td>LEB</td> <td>NIEHS</td> </tr> </table>			PI:	Teruzo Konishi	Visiting Scientist	LEB	NIEHS	Other:	Alec Salt	Visiting Fellow	LEB	NIEHS		Philip E. Hamrick	Senior Scientist	RRP	NIEHS		Edward Lebetkin	Biological Technician	LEB	NIEHS		Charles Hamm	Physical Science Tech.	LEB	NIEHS
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COOPERATING UNITS (if any) Research Resources Programs																											
LAB/BRANCH Laboratory of Environmental Biophysics																											
SECTION Noise Effects Research																											
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																											
TOTAL MANYEARS: 1.3	PROFESSIONAL: .9	OTHER: .4																									
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																											
SUMMARY OF WORK (200 words or less - underline)																											
The long range purpose of this project is to increase our understanding of the basic mechanisms of <u>electrolyte transport</u> in the <u>guinea pig cochlea</u> under normal conditions and under the influence of physical and chemical agents. The aim of the current project is (1) to reveal electrolyte movements (Na^+, K^+ and Cl^-) across the <u>endolymph-perilymph barrier</u> after exposure to intermediate levels of noise and (2) to correlate alteration of the <u>ionic permeability</u> of the cochlear partition with changes in sensitivity of the <u>auditory system</u>.																											

PROJECT DESCRIPTION

METHODS EMPLOYED: Healthy guinea pigs were exposed to wide band white noise for seven days (16 hours on and 8 hours off per day). The overall intensities were 95, 105 or 115 dBA. After the period of noise exposure the cochlear potentials were recorded under anesthesia. The perilymphatic space was perfused with artificial perilymph containing ^{43}K , ^{22}Na or ^{36}Cl . Concentrations of Na^+ and K^+ in the perfusate, perilymph and endolymph were determined by a helium-glow photometer and activities of these radioisotopes were measured using a gamma spectrometry system. Concentration of Cl^- in the fluid samples were measured by a microtitration method and the activities of ^{36}Cl were determined using a liquid scintillation counter. The degree of hearing loss resulting from exposure to noise was estimated by monitoring the brainstem evoked responses from guinea pigs with chronically implanted electrodes at the vertex. The evoked responses were averaged in a computer and the intensity-latency functions were determined.

MAJOR FINDINGS AND PROPOSED COURSE:

1. EFFECT OF NOISE

1. The brainstem evoked responses to clicks were rapidly suppressed and reached the asymptotic level during the first and second day of exposure to noise at 105 dBA. There was a slight recovery of the evoked responses during the off-period but as the exposure was prolonged, the sensitivity of evoked responses remained around 60 dB above the pre-exposure level. There was considerable recovery of the brainstem responses after the 7 days exposure to noise was terminated.

2. The cochlear microphonics (CM) were suppressed by 7 days exposure to noise at 95, 105 or 115 dBA. The loss of CM sensitivity (expressed by lateral shift in dB of the CM input-output function) and suppression of the maximum CM output averaged 23 dB and 13.5 dB respectively at 115 dB exposure level. At 105 dBA exposure level they were 12 dB and 7.9 dB respectively. The action potential in the auditory nerve (AP) was more susceptible to noise than the CM. After 115 dBA exposure the AP could not be observed when 6 KHz tone bursts were presented at 100 dB SPL. After 105 dBA exposure the AP was only elicited by test tones of higher than 80 dB SPL intensity.

3. The endocochlear potential (EP) recorded from noise exposed animals was 86.0 ± 2.7 mV, 89.1 ± 3.4 mV and 84.5 ± 4.3 mV at 95, 105 and 115 dBA levels of noise respectively. These magnitudes of the EP were not substantially different from controls (83.2 ± 2.2 mV).

4. The total concentrations of Cl^- in the endolymph and perilymph were not affected by noise exposure. The concentration ratio between the endolymph and perilymph was 0.994 ± 0.032 and 0.984 ± 0.032 at 105 dBA and 115 dBA exposure levels respectively and these ratios were not statistically different from values obtained from the non-exposed guinea pigs.

5. The rate of uptake of ^{36}Cl into the endolymph was not significantly affected by noise exposure. The transport rate constants were 0.011 and 0.009 at 105 and 115 dBA exposure levels and these values were comparable with that of 0.011 in the non-exposed group.

6. The total concentrations of K^+ and Na^+ in the endolymph of the noise exposed guinea pigs (noise level 105 dBA) were 159.6 ± 5.36 mM and 0.68 ± 0.44 mM respectively and did not show a statistical difference from the control values.

7. The rate of uptake of ^{43}K into the endolymph was suppressed by exposure to noise at 105 dBA. There was considerable variation in the transport rate constants but the main group of noise exposed animals showed the rate constant of 0.0075 whereas the rate constant in the control animals was 0.0126.

2. EFFECT OF ETHACRYNIC ACID.

When the perilymphatic space was perfused with ^{36}Cl solution containing 200 μM ethacrynic acid, the sound-evoked responses and the EP were greatly decreased and the EP became negative 20 minutes after commencement of the perfusion. The total concentration of Cl^- in the endolymph decreased and the Cl^- concentration ratio between the endolymph and perilymph ranged from 0.88 to 0.90 forty minutes after perfusion commenced. The rate of uptake of ^{36}Cl into the endolymph was also reduced. The suppression of ^{36}Cl uptake observed during perfusion with ethacrynic acid was quite similar to that observed during perfusion with ouabain.

We plan to perform (1) systematic studies in an effort to reveal ototoxicity of diuretics which may occur as a result of the disturbance of the dynamic equilibrium of electrolytes in the cochlear fluids; (2) studies of electrolyte movement across the endolymph-perilymph barrier under hypothermic conditions and (3) Study of combined effects of noise and chemical agents (ototoxic chemicals) or physical agents (temperature).

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The nature of the permeability of the endolymph-perilymph barrier to various electrolytes has been frequently hypothesized but the supporting data are rather limited. Present data suggest the possibility that transport of K^+ across the cochlear partition is involved in the pathophysiological processes underlying the noise-induced hearing loss. These studies are a part of our efforts to increase our understanding of the disturbance of the inner ear under the influence of physical and chemical agents.

PUBLICATIONS

Konishi, T., Hamrick, P.E., and Walsh, P.J.: Ion transport in guinea pig cochlea. 1. Potassium and sodium transport. Acta Otolaryngol. (Stockholm), in press.

Konishi, T. and Hamrick, P.E.: Ion transport in guinea pig cochlea. 2. Chloride transport. Acta Otolaryngol. (Stockholm) in press.

Chen, G. and Konishi, T.: A simple current injection: Its application to coulomb titration of chloride. Med. Biol. Engineering Computing, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50014-09 LEB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Microwave Exposure Systems and Microwave Dosimetry		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Donald I. McRee Research Physicist LEB NIEHS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Environmental Biophysics		
SECTION Microwave Research		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 1.1	PROFESSIONAL: 0.2	OTHER: 0.9
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (a1) MINORS ☐ (a2) INTERVIEWS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER		
SUMMARY OF WORK (200 words or less - underline)		
It is the objective of this project to develop <u>exposure systems</u> for <u>bioeffects research</u> and to develop and test techniques for measuring <u>energy absorption</u>. The <u>waveguide system</u> for exposing isolated neurons to 2450 MHz microwave radiation has been modified so that longer survival times and constant temperatures of the nerves during exposure could be achieved. Small thermistor probes have been used to measure deep colonic temperatures in pregnant mice during exposures to 2450 MHz microwave radiation. Using the temperature profiles measured in the dead animals to calculate average absorption rates, the results agreed well with both calculated and measured whole body absorption reported in the literature. A system for exposing groups of marine animals to different intensity levels simultaneously without significant perturbation of the microwave field by the location and orientation of the animals has been developed and tested.		

PROJECT DESCRIPTION

METHODS EMPLOYED: The objectives of this project are to develop microwave exposure systems for biological material which have well-defined, field characteristics; and to develop dosimetric techniques for energy absorption. A thorough study of the research requirements on the biological effects of microwaves and techniques for exposing biological specimens have been completed. Based on these studies, exposure systems will be designed and developed which provide known electromagnetic field characteristics. At present, it is difficult to accurately measure low level microwave fields and to determine the mechanisms by which the fields interact with matter. It is necessary, therefore, to conduct dosimetric studies so as to develop analytical interaction models. Microcalorimetry, thermistor and thermocouple detectors, and liquid crystals are some of the techniques which will be investigated. Other techniques to determine energy absorption will be evaluated as they become available.

MAJOR FINDINGS AND PROPOSED COURSE: It has been determined that for the exposure of small biological specimens such as cell systems and isolated neurons, waveguide exposure systems offer excellent characteristics. A waveguide system for exposing isolated neurons to 2450 MHz microwave radiation has been modified and improved. One modification permits the use of mineral oil in the neural stimulating and recording electrode modules so that longer survival times of the isolated nerves can be achieved. A second modification allows the circulation of saline through a constant temperature water bath into the waveguide exposure chamber. This modification enables us to maintain a constant temperature of both the control and exposed nerves during various power density levels of irradiation.

Small thermistor probes fabricated in our laboratory were used to measure the deep colonic temperatures in pregnant mice during exposure to 2450 MHz microwave radiation. Using these temperature profiles to calculate an average specific absorption rate for the whole body, the results agreed well with both calculated and experimental whole body absorption determined by investigators at the University of Utah and University of Washington.

A system for exposing marine animals was designed, fabricated and tested. The system can be used to expose multiple animals to different intensity levels simultaneously without significant perturbation of the field by the location and orientation of the animals.

Waveguide exposure systems will continue to be fabricated, calibrated, and operated during the next fiscal year. The new systems will provide the Institute with the capability of exposing isolated neurons, cell systems and macromolecules to not only continuous wave, but other wave forms such as pulsed and frequency modulated waves. In addition, the new system will allow us to expose isolated mammalian nerves which are difficult to maintain in a viable state outside the animal body. The development of a system to measure the

electrical properties, dielectric constant and conductivity, of biological material will continue. A small thermistor probe, 1 mm in diameter, with projected accuracy of $\pm 0.01^{\circ}\text{C}$ and excellent response time has been developed by the National Bureau of Standards. This probe is said to have no interaction problems with microwave radiation. We have requested one of these probes for testing in our laboratory.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The development of exposure system with well-defined field characteristics will enable the Institute to perform accurate quantitative studies on the effects of microwave radiation on biological systems at frequencies ranging from 1-10 GHz. Techniques to determine the amount of energy absorbed will provide the capability of evaluating the data in terms of thermal or specific microwave effects.

PUBLICATION

McRee, D.I. and Wachtel, H.: Long-term, low-level exposure of marine animals. Radio Science, (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50015-05
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Effects of Microwaves on Neural Response		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Donald I. McRee Other: Howard Wachtel Research Physicist Associate Professor LEB Duke U. NIEHS		
COOPERATING UNITS (if any) Duke University, Durham, North Carolina		
LAB/BRANCH Laboratory of Environmental Biophysics		
SECTION Microwave Research		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.8	PROFESSIONAL: 0.4	OTHER: 0.4
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline 4. The objective of this project is to determine the effect of <u>microwave radiation</u> on <u>neurological response</u> . Isolated neurons such as the abdominal ganglion of the <u>Aplysia</u> , the sciatic nerves of frogs and the saphenous nerves of cats will be exposed to CW, pulsed and modulated microwave radiation in the power density range of 1-10 mW/cm ² . The effects of the microwave radiation on the <u>strength-duration</u> of a stimulus to produce an <u>action potential</u> , amplitude of the response, and conduction velocity will be investigated. The fatigue and recovery of the neurons under rapid, multiple stimulation will also be studied. A series of experiments to determine if continuous wave, 2450 MHz microwave radiation increases the fatigue rate or changes the vitality of the frog sciatic nerve were completed. The frog sciatic nerves were exposed in a waveguide to specific absorption rates of 100 W/kg, 50 W/kg, and 20 W/kg. The nerves were stimulated with twin pulses (separated by 5 ms interval) at a repetition rate of 500 pps in order to accelerate the rundown time. Distinct changes in the excitability and refractoriness of the exposed nerve were seen in comparison to the control nerve. The vitality of the nerve was affected and was greater for the higher dose rates.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Isolated neurons such as the abdominal ganglion of the Aplysia, the sciatic nerve of the frog, and the saphenous nerve of cats will be exposed to CW, pulsed and modulated microwave radiation in the power density range of 1-10 mW/cm². The effects of the radiation on cell membrane potentials, action potentials and conduction velocity will be investigated.

More complex systems such as the exposed spinal cord of decerebrated cats will be exposed. These test systems will be used to investigate the effects of radiation on synaptic neurotransmitters.

MAJOR FINDINGS AND PROPOSED COURSE: A review of the literature indicates that the brain and central nervous system are sensitive to microwave radiation. In most studies, no explanation of the mechanism by which microwaves interact with nerve cells is specified. It has been determined that the best technique for exposing isolated neurons to microwaves, without interaction of the microwaves with the electrodes, is in a waveguide system. A waveguide exposure system has been designed and built. Equipment for stimulation and recording of neural responses has been assembled.

The waveguide exposure system has been modified so that the nerve can be maintained over a longer period of time and kept during exposure at a constant temperature. Experiments will be repeated to evaluate the effects of temperature and to determine thresholds for the effect on this test system. It is believed that the effect observed to this point are due to changes in active transport across the neural membrane. Labeled ions will be used to measure changes in ionic transport in order to attempt to explain the mechanism of action producing the change in vitality of the exposed nerve. Exposure of the nerves to other waveforms (pulsed and modulated) will be carried out in order to determine if they are more or less effective in producing the observed effect.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The potential health effects of microwave radiation in the environment is of interest to NIEHS. The neurological and behavioral effects reported in the literature illustrate the need for significant effort in this area. At present, the accepted safe level of exposure in the U.S. is 1000 times greater than the standard in the USSR. The Soviet standard is based on neurological and behavioral response to microwave radiation. This research on the effects of microwave on CNS is directed toward the mission of the Institute to determine the health effects of physical factors in the environment.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50016-07 LEB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Effects of 2450 MHz Microwave Radiation on Biological Materials at Cellular Level		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Philip E. Hamrick Donald I. McRee Senior Scientist Research Physicist RRP NIEHS LEB NIEHS		
COOPERATING UNITS (if any) Research Resources Program		
LAB/BRANCH Laboratory of Environmental Biophysics Branch		
SECTION Microwave Research		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline < The objectives of this project are to determine how <u>2450 MHz microwave radiation</u> interacts with biological material at the cellular and macromolecular level, to observe any effects of this interaction and to relate the amount of microwave energy absorbed to the effects. Biological systems employed in the study are: <u>bacteria and bacteriophage</u>, solutions of <u>DNA</u>, 2-4 cell stage <u>embryos</u> from mice, <u>rabbit erythrocytes</u> and <u>rat lymphocytes</u>. In these experiments, the temperature, humidity, and other various growth conditions have been carefully monitored in order to eliminate any effects not intrinsically caused by the microwave radiation. Results have shown very few differences between exposed and control samples that could not be explained by the thermal heating of the microwave radiation.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Various cellular and macromolecular systems are exposed to microwave radiation at carefully controlled temperatures and exposure levels. Much of the work has been at 37°C. Biological systems employed in the study are: bacteria and bacteriophage, solutions of DNA, 2-4 cell stage embryos from mice, rabbit erythrocytes and rat lymphocytes.

MAJOR FINDINGS AND PROPOSED COURSE: During the past year our work has been restricted to completing a study of the effect of 2450 MHz CW radiation on rat lymphocytes in culture. This work has shown that there is no significant difference in stimulation when cells are exposed for 40 hours or less to 5, 10, or 20 mW/cm² of 2450 MHz CW microwave radiation and in cells not exposed but held under similar conditions of temperature and humidity.

We plan to terminate this project since the scope of the project is too broad. New projects of more specific scope will be initiated in which sensitivity to microwave radiation has been reported or which are desirable test systems.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Before an accurate evaluation of the biological effects of 2450 MHz microwaves can be made, it is necessary to carefully control the temperature of the specimen and be able to reproduce the exposure conditions. We have the capability to do this at NIEHS and may distinguish observed effects of microwaves from purely thermal effects.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50017-05 LEB															
PERIOD COVERED October 1, 1977 to September 30, 1978																	
TITLE OF PROJECT (80 characters or less) Effects of 2450 MHz Microwaves on Embryonic Development, Immunology and Fertility																	
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																	
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI:</td> <td style="width: 33%;">Donald I. McRee</td> <td style="width: 33%;">Research Physicist</td> <td style="width: 10%;">LEB</td> <td style="width: 10%;">NIEHS</td> </tr> <tr> <td>Others:</td> <td>J.P. Thaxton</td> <td>Consultant</td> <td>NCSU</td> <td></td> </tr> <tr> <td></td> <td>C.R. Parkhurst</td> <td>Consultant</td> <td>NCSU</td> <td></td> </tr> </table>			PI:	Donald I. McRee	Research Physicist	LEB	NIEHS	Others:	J.P. Thaxton	Consultant	NCSU			C.R. Parkhurst	Consultant	NCSU	
PI:	Donald I. McRee	Research Physicist	LEB	NIEHS													
Others:	J.P. Thaxton	Consultant	NCSU														
	C.R. Parkhurst	Consultant	NCSU														
COOPERATING UNITS (if any) Poultry Science Department, North Carolina State University																	
LAB/BRANCH Laboratory of Environmental Biophysics																	
SECTION Microwave Research																	
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																	
TOTAL MANYEARS: 1.3	PROFESSIONAL: 0.6	OTHER: 0.7															
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																	
SUMMARY OF WORK (200 words or less - underline) The objectives of this research project is to study the effects of microwave radiation on embryonic development, and immunology and fertility of the mature quail which were exposed during development. Fertilized Japanese quail eggs were exposed to 2450 MHz microwave radiation at incident power density of 5 mW/cm ² for the first 12 days of embryogenesis. The eggs were then transferred to a regular hatching incubator until hatch (17 days). Control eggs were treated exactly the same way except for the microwave exposure. At hatch the quail were checked for deformities. No differences were found in the hatchability or development in the controls and exposed quail. The quail were then placed in regular feeding and housing facilities and were not exposed to any microwave radiation after hatch. Reproductive performance in the quail was monitored from 6 through 22 weeks of age. Non-exposed controls of both sexes, as well as exposed females, performed normally. However, the exposed males did not. This conclusion is based upon reduced sperm numbers, reduced sperm motility, and a decreased percentage of fertile eggs produced by control or exposed females when paired with exposed males. Mating behavior was normal in both exposed and non-exposed males.																	

PROJECT DESCRIPTION

METHODS EMPLOYED: The objectives of this project are to determine the effects of 2450 MHz CW microwave radiation on the embryological development of Japanese quail and the subsequent growth, reproduction, and immunological response of the mature quail which had been exposed during the developmental period. Fertilized Japanese quail eggs were exposed to 2450 MHz CW microwave power at an incident power density of 5 mW/cm^2 for the first 12 days of embryogenesis. The effects of the exposure on hatchability and gross deformities were measured in the two day old quail. The quail were kept to maturity and growth rates, egg production, fertility and immunological response were measured.

MAJOR FINDINGS AND PROPOSED COURSE: Exposure of the fertilized eggs to a power density of 5 mW/cm^2 for the first 12 days of embryogenesis were completed. The eggs were then transferred to a regular hatching incubator until hatch (17 days). Control eggs were treated exactly the same way except for the microwave exposure. At hatch the quail were checked for deformities. No differences were found in the hatchability or development in the control and exposed quail. The quail were then placed in regular feeding and housing facilities and were not exposed to any microwave radiation after hatch. Reproduction performance in the quail was monitored from 6 through 22 weeks of age. Non-exposed controls of both sexes, as well as exposed females, performed normally. However, the exposed male did not. This conclusion is based upon reduced sperm numbers, reduced sperm motility, and a decreased percentage of fertile eggs produced by control or exposed females when paired with exposed males. Mating behavior was normal in both exposed and non-exposed males.

The proposed course of research will be to verify the fertility studies and to continue the studies to determine the effects of this exposure on immunological response. Previous studies have indicated that the bursa and spleen weights are different in the exposed quail at the 22 week old age. Foreign cell types will be injected into the 22 week old quail and antibody production will be measured. The effects of microwave exposure during embryogeny on longevity will also be investigated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The evaluation of whether exposure to microwaves during embryonic development has an effect on the development process and function of the organism after maturity must be answered before microwave radiation exposure can be considered safe. This project will assist the Institute in its mission of determining the health effects of physical factors in the environment.

PUBLICATIONS

McRee, D.I. and Hamrick, P.E.: Exposure of Japanese quail embryos to 2.45 GHz microwave radiation during development. Radiation Research 71: 355-366 (1977).

Hamrick, P.E., McRee, D.I., Thaxton, J.P. and Parkhurst, C.R.: Humoral immunity of Japanese quail subjected to microwave radiation during embryogeny. Health Physics 33: 23-33, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50019-03 LEB															
PERIOD COVERED October 1, 1977 to September 30, 1978																	
TITLE OF PROJECT (80 characters or less) Effect of Ototoxic Insult on Coding of Complex Signals in the Auditory System																	
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">Teruzo Konishi</td> <td style="width: 25%;">Visiting Scientist</td> <td style="width: 10%;">LEB</td> <td style="width: 15%;">NIEHS</td> </tr> <tr> <td></td> <td>Reginald O. Cook</td> <td>Acoustical Engineer</td> <td>LEB</td> <td>NIEHS</td> </tr> <tr> <td>Others:</td> <td>Charles W. Hamm</td> <td>Physical Science Tech.</td> <td>LEB</td> <td>NIEHS</td> </tr> </table>			PI:	Teruzo Konishi	Visiting Scientist	LEB	NIEHS		Reginald O. Cook	Acoustical Engineer	LEB	NIEHS	Others:	Charles W. Hamm	Physical Science Tech.	LEB	NIEHS
PI:	Teruzo Konishi	Visiting Scientist	LEB	NIEHS													
	Reginald O. Cook	Acoustical Engineer	LEB	NIEHS													
Others:	Charles W. Hamm	Physical Science Tech.	LEB	NIEHS													
COOPERATING UNITS (if any) None																	
LAB/BRANCH Laboratory of Environmental Biophysics																	
SECTION Noise Effects Research																	
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																	
TOTAL MANYEARS: 1.35	PROFESSIONAL: .65	OTHER: .70															
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																	
SUMMARY OF WORK (200 words or less - underline < Results of <u>behavioral and audiological tests</u> with humans imply that ability to understand <u>speech and other complex signals</u> (particularly in a noisy listening situation) suffers loss from noise insult in excess of what could be inferred from pure tone thresholds measured in the quiet. These findings suggest that a <u>complex interaction</u> between the various levels of the auditory nervous system and functionally different receptors of the auditory end organ play a vital role in "sharpening" the <u>sensory process</u> . The objective is to use the rapid signal analysis ability of minicomputers to determine the auditory response to speech and speech-like signals including pseudo-random noise under normal conditions and conditions of auditory fatigue. Auditory fatigue and recovery process of single nerve fiber to speech and speech-like stimuli will be studied. The requirement in high speed data throughput for nearly absolute phase matching of input acoustical and output physiological signals, and for accurately processing neural pulse trains necessitated the design and building of several unique complex peripheral devices. These are presently being debugged.																	

PROJECT DESCRIPTION

METHODS EMPLOYED: In order to most efficiently produce speech-like inputs, pseudorandom noise which has been amplitude modulated by sinusoidal signals is presented to the animal. The pseudorandom noise approximates the constantly changing frequencies of speech while the sinusoidal modulation simulates the varying intensities. When the sinusoidally modulated pseudorandom stimulus is presented to the animal the auditory nerve response is simultaneously recorded in the form of a cycle histogram, the cycle being locked to the period of pseudorandom noise. After summing the histogram many times to ensure statistical validity, cross-properties between the output and the known input, which represent the response characteristic of the peripheral auditory system can be determined.

1. In order to obtain the temporal characteristics of the input noise, a high-speed analog-to-digital converter (ADC) system that can convert at speeds up into the range of 40-50 KHz was necessary. Three channels (at roughly the same high speed) are needed for this and other planned experiments. Since no commercially available ADC system with a PDP-11 interface with sufficiently high speed existed, it was necessary to design and construct one around commercially available high-speed ADC modules and interfacing boards. The decision was a cooperative project between computer engineering, Biometry Branch, our electronic consultant, and LEB personnel.

2. For acquiring the histogram data, a special high-speed clocked pulse counter interfaced directly with the computer was designed by and built on a commercially available interface board by LEB personnel with design guidance from computer engineering, Biometry Branch. As with the ADC, the special design was primarily necessitated by the 40-50 KHz data rate.

3. As an intrinsic adjunct to any high-speed ADC system, a low-pass sharp-cutoff filtering system must be provided in order to prevent aliasing of high frequencies. Phase matching requirements of such filtering systems when time dependent cross properties are to be measured far exceed those normally available filters. State of the art elliptic filters providing a higher maximum data rate and increased phase accuracy became available last year and were built into the system by LEB personnel.

We are presently at the stage of verifying the correctness of software algorithms by inserting multiple signals whose cross properties are known.

MAJOR FINDINGS AND PROPOSED COURSE: Major progress has been in the design and construction of state of the art computer interface devices described above. Pilot projects involving normal animals to acquire baseline data and establish the resolution capability of the system will proceed followed by subsequent testing of noise exposed animals.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In recent years, it has become clear that the ability of the cochlear transducer

to respond to pure tones is not an adequate indicator of its ability to respond to the natural complex stimuli present in the real world. Studies of the former are legion, studies in the latter area, particularly after ototoxic insult, are nearly nonexistent in spite of the fact that complex stimuli perceived in a noisy environment constitute the natural situation. We hope to obtain data which will begin to fill this void.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50020-03 LEB																
PERIOD COVERED October 1, 1977 to September 30, 1978																		
TITLE OF PROJECT (80 characters or less) Optical Fiber Motion Detector for Auditory System Measures																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																		
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 45%;">Reginald O. Cook</td> <td style="width: 20%;">Acoustical Engineer</td> <td style="width: 20%;">LEB NIEHS</td> </tr> <tr> <td>Others:</td> <td>Adnan Akay</td> <td>Visiting Fellow</td> <td>LEB NIEHS</td> </tr> <tr> <td></td> <td>Charles W. Hamm</td> <td>Physical Science Tech.</td> <td>LEB NIEHS</td> </tr> <tr> <td></td> <td>Teruzo Konishi</td> <td>Visiting Scientist</td> <td>LEB NIEHS</td> </tr> </table>			PI:	Reginald O. Cook	Acoustical Engineer	LEB NIEHS	Others:	Adnan Akay	Visiting Fellow	LEB NIEHS		Charles W. Hamm	Physical Science Tech.	LEB NIEHS		Teruzo Konishi	Visiting Scientist	LEB NIEHS
PI:	Reginald O. Cook	Acoustical Engineer	LEB NIEHS															
Others:	Adnan Akay	Visiting Fellow	LEB NIEHS															
	Charles W. Hamm	Physical Science Tech.	LEB NIEHS															
	Teruzo Konishi	Visiting Scientist	LEB NIEHS															
COOPERATING UNITS (if any) None																		
LAB/BRANCH Laboratory of Environmental Biophysics																		
SECTION Noise Effects Research																		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																		
TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.3	OTHER: 0.1																
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (a1) MINORS ☐ (a2) INTERVIEWS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER																		
SUMMARY OF WORK (200 words or less - underline keywords) A convenient means for making audio frequency displacement measures of the ossicular chain at displacement levels (1-100 Angstrom) corresponding to normal sound levels (40-80 dB SPL) has long been needed in the study of auditory distortion and hearing loss mechanisms. Reliable measures of the motion of the basilar membrane would resolve present uncertainties concerning non-linear plateau effects at high intensities and frequency resolution from high and low frequency slopes of the maximas which block confirmation of hearing theories at the intra cochlear mechanical level. Developmental work in the past year has led to the construction of very compact laser energized fiber optic levers with which instantaneous waveform visualization at the 10 Angstrom level can be accomplished on ossicular chain preparations; the electro optic technology has been advanced far enough to be released for general auditory research use. This optic lever system is competitive with interferometric and optical heterodyning techniques in resolution and is an order of magnitude simpler and less expensive.																		

PROJECT DESCRIPTION

METHODS EMPLOYED: Physics, electro optics, optics (fibers), electronic engineering, computer modeling.

MAJOR FINDINGS AND PROPOSED COURSE: The fiber optic lever, although an intrinsically powerful vibration and pressure measuring technique, is characterized by approximately 7 different parameters. To realize the full potential of the lever, these parameters must be optimized for any given application. The vibratory movements of the auditory system at normal speech levels are so low (1-100 A) and cover such wide bandwidth that optimized performance of even powerful levers is necessary. An analysis of these parameters when the layer is energized with collimated (laser) or uniaxial light was previously completed and verified in FY 77. Analysis and verification was completed for the more complex case of illumination with noncollimated sources in FY 78. The latter analysis showed that for most cases simpler to operate nonlaser sources were superior, and additionally revealed the exact optimum design for ossicular chain applications, a design with which the sought 1-100 A resolution at 10 KHz BW can be achieved. The analysis and verification revealed that commercially available electronic components and fibers did not compromise lever capabilities (compared with ideal requirements). At present, very high radiance tungsten lamps are slightly better than light emitting diodes as illumination sources but with the latter fast gaining as result of research advances by the communications industry. The development phase of project is complete except for attempts to find better commercially available illumination sources. The problem with making basilar membrane measurements has been reduced to one of fabrication of a fiber bundle of extremely small size from whose tip the light can be turned 90° in an extremely small space. The latter is being pursued with several fiber optic specialty manufacturers at present.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In addition to this auditory system application, optic levers have been utilized to measure the dynamic force exerted by muscle myofibrils, as the basis for ultra-sensitive pressure transducers for cardiovascular and other internal purposes, and for non-contacting measurement of the intra-ocular pulse. Although the non-contacting characteristic of optic levers uniquely qualify them for biological (and many mechanical) applications, application of the technique has previously suffered from lack of a theoretical basis from which design optimization could proceed, and from the relatively short working distances and low detection limits associated with the commercially available models. Since the theoretical relationships have been developed for both laser and Lambertian sources and confirmed by measurement, it is now possible to accurately predict the ultimate resolution for given fibers, bundle configuration and illumination source, thus making possible significant real advances in the state of the art, as well as providing an optimized transducer to solve a specific problem.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50022-02 LEB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Development of Impact Noise Generator for Hearing Loss Studies		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Adnan Akay Other: Reginald O. Cook	Visiting Fellow Acoustical Engineer	LEB LEB NIEHS NIEHS
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Environmental Biophysics		
SECTION Noise Effect Research		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.40	PROFESSIONAL: 0.30	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline) The purpose of this project is to design and build an <u>impact noise generator</u> whose peak level, rise time, and decay can be experimentally manipulated so that the effects of these parameters on hearing loss can be studied in laboratory animals. Lack of such a generator has greatly contributed to the paucity of controlled laboratory studies of <u>impact noise effects on hearing</u> . Because of the high instantaneous peaks and rise times of impact noise, it cannot be recorded and reproduced in the conventional manner with audio equipment.		

PROJECT DESCRIPTION

METHODS EMPLOYED: In order to simulate exposure to impactive noise existing in industrial situations various impact noise generation mechanisms were investigated analytically and experimentally to determine the vital characteristics of the radiated sound pulses, such as rise time, frequency content, energy level, peak pressure level, repetition rate, etc. Finally, based on these findings, a prototype impact sound generator was designed for use in laboratory noise exposure studies. The motive source chosen for this generator was a very large solenoid, which allows impact repetition rate manipulations to a much greater extent than other mechanical devices. The impact of the solenoid is actuated by a specially designed electronic circuit to control duration of each impact, applied voltage, which in turn determines the speed, and finally the repetition rate of the impacts.

MAJOR FINDINGS AND PROPOSED COURSE: The four basic impact sound generation mechanisms were found to be air ejection from between the colliding surfaces, radiation due to acceleration of the impacting rigid bodies, radiation due to rapid surface deformations caused by collisions, and pseudo-steady state radiation (ringing) of the impacted object. Except under ideal conditions, the sound radiation from the impact of two or more solid bodies is a result of a combination of several of these mechanisms. To generate the necessary fast rise-time and high peak pressure levels, several "impact heads" were designed, whose geometric configuration determined the nature of the radiated sound pulse. To obtain the necessary force, speed and the ability to control the repetition rate of impacts a large solenoid was selected. However, to insulate the consequent high magnetic fields required extensive design changes. A final model is in production stage. It is suggested that this model is tested in laboratory exposure studies and made more practical to operate and transport.

The sound pressure levels produced by the designed prototype "impact heads" reach 140 dB which is well in the range necessary to simulate industrial exposures of 120-160 dB peak.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The fact that it is not possible to reproduce impact sounds using conventional audio equipment has resulted in few if any well controlled laboratory studies of hearing damage from a type of noise which pervades industry and society. There is strong suggestive evidence that high intensity short rise time peaks rather than steady state levels are the major contributor to hearing loss. Because the temporal summing characteristics of the human auditory system operate over a longer time span than that of impact sounds, perceived loudness is not proportional to peak level or to peripheral organ damage possibilities. This effort should make refined studies of hearing loss/impact noise parameters possible.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50023-02 LEB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Review of Impact Noise, its Mechanism, Control and Effects		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Adnan Akay Visiting Fellow LEB NIEHS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Environmental Biophysics		
SECTION Noise Effects Research		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (a1) MINORS ☐ (a2) INTERVIEWS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER		
SUMMARY OF WORK (200 words or less - underline) This project consists of a review of the literature on impact noise. Its purpose is to increase the understanding of basic mechanisms of impact sound generation, impact noise control and to review the status of present and proposed damage assessment criteria resulting from impact noise exposure.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Literature review and critical evaluation.

MAJOR FINDINGS AND PROPOSED COURSE: The mechanisms of impact sound generation are classified as air flow ejected from the space between rapidly approaching surfaces, rigid-body radiation, radiation from rapid surface deformations, pseudo-steady state radiation and as a consequential source, radiation from material fracture. These methods have been investigated recently but the present theoretical tools are not sufficient to describe the impact sound radiation in detail.

Although hearing loss due to impact noise has been reported as early as 1700, the significance of high intensity fast rise-time impulse sound has been long overlooked. The present status of research results does not appear sufficient for an understanding of the parameters or to support standards for impact noise induced hearing damage assessment.

Project is terminated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The effect of impactive impulse sounds on hearing have not until very recently been recognized as a probable major source of hearing damage. This review summarizes the available work on the subject and emphasizes the need to consider this long neglected phenomenon.

PUBLICATIONS

Akay, A.: A Review of Impact Noise. J. Acoust. Soc. Amer., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50024-01 LEB						
PERIOD COVERED October 1, 1977 to September 30, 1978								
TITLE OF PROJECT (80 characters or less) Effects of Microwaves on Sister Chromatid Exchange in Bone Marrow Cells of Mice								
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT								
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Donald I. McRee</td> <td style="width: 33%;">Research Physicist</td> <td style="width: 33%;">LEB NIEHS</td> </tr> <tr> <td>Other: Gordon Livingston</td> <td>Postdoctoral</td> <td>U. of Utah</td> </tr> </table>			PI: Donald I. McRee	Research Physicist	LEB NIEHS	Other: Gordon Livingston	Postdoctoral	U. of Utah
PI: Donald I. McRee	Research Physicist	LEB NIEHS						
Other: Gordon Livingston	Postdoctoral	U. of Utah						
COOPERATING UNITS (if any) None								
LAB/BRANCH Laboratory of Environmental Biophysics								
SECTION Microwave Research								
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709								
TOTAL MANYEARS: 0.35	PROFESSIONAL: 0.1	OTHER: .25						
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS								
SUMMARY OF WORK (200 words or less - underline key words) The objective of the project was to investigate the effects of whole body irradiation of the mouse on the number of <u>sister chromatid exchanges</u> in bone marrow cells. The measurement of sister chromatid exchange (SCE) constitutes the most sensitive indicator yet developed for detecting cytogenetic effects of mutagens and carcinogens. We utilized this sensitive assay to investigate potential mutagenic bioeffects of 2450 MHz CW microwave radiation by comparing the incidence of SCE in bone marrow cells of 12 exposed mice, 12 sham controls, and 12 standard controls. The animal were exposed from above for 8 hours/day to an incident power density of 20 mW/cm ² (SAR determined to be 15.4 mW/gm). The exposure was for 28 days. Immediately after termination of exposure, the animals were prepared for analysis. Results after scoring more than 300 cells show that the incidence of SCE's in the exposed group is not significantly different from the unexposed controls. The value is approximately 3.0 exchanges per cell.								

PROJECT DESCRIPTION

METHODS EMPLOYED: The measurement of sister chromatid exchange (SCE) constitutes the most sensitive indicator yet developed for detecting cytogenetic effects of mutagens and carcinogens. We utilized this sensitive assay to investigate potential mutagenic bioeffects of microwave (2450 MHz) radiation by comparing the incidence of SCE in bone marrow cells of 12 exposed mice, 12 sham controls, and 12 standard controls following a 28 day treatment period. Animals were exposed from above using an absorber-lined horn irradiator under which 12 styrofoam cages were oriented in a circular geometry so that uniform field exposure could be achieved for each mouse. The daily treatment regime consisted of two four hour exposure periods between which one hour was provided at midday when animals were fed and watered while the horn antenna was turned off. The incident power density, as measured with an NBS isotropic probe, inside the styrofoam cages ranged from 18 to 20 mW/cm². At a level of 20 mW/cm² the specific absorption rate was calculated to be 15.4 mW/cm. Measurement of SCE frequency was performed by differential labelling of sister chromatids with the base analog 5-bromodeoxyuridine and subsequent staining of chromosome preparations with Hoechst 33258 and Giemsa. All 36 animals were injected with 10⁻² M BrdU at intervals of 45 minutes over an eight hour period. At 19 1/2 hours after the first injection colchicine was administered IP to arrest cells in metaphase. After 90 minutes bone marrow cells were harvested from both femurs using standard cytogenetic procedures. Air dried slides were stained with the Fluorescent Plus Giemsa method to visualize SCE's. Slides were randomized, coded and then scored.

MAJOR FINDINGS AND PROPOSED COURSE: After scoring over 300 cells, results show that the number of SCE's per cell in the microwave exposed group is not significantly different from the unexposed controls. The value is approximately 3.0 exchanges per cell. From these results it would appear that no mutagenic effect occurs in mouse bone marrow cells when exposed to 2450 MHz microwave radiation at a power density of 20 mW/cm². Although it would be desirable to use this test system for other microwave frequencies, power densities, and waveforms (pulsed and modulated) no future work is planned at this time.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Whether microwave radiation does or does not produce mutagenic effects has not been adequately tested. With the rapid growth of microwave sources, more people are being exposed to low-level microwave energy. This project has contributed to the evaluation of the biological significance of this exposure and contributes to the mission of the Institute to determine the biological effects of physical factors on man and the environment.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50025-01 LEB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) The Effect of Microwave Radiation on the Heart Rate of Embryonic Quail		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Philip E. Hamrick Other: Donald I. McRee Senior Scientist RRP Research Physicist LEB NIEHS NIEHS		
COOPERATING UNITS (if any) Research Resources Program		
LAB/BRANCH Laboratory of Environmental Biophysics		
SECTION Microwave Research		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.3	PROFESSIONAL: 0.3	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline) The objectives of this project are to determine if and how <u>microwave radiation</u> interacts with the <u>embryonic quail heart</u> or causes a change in heart rate. If a heart rate effect is found to exist, the change in <u>heart rate</u> as a function of absorbed microwave energy will be studied. Changes in heart rate due to temperature changes will be controlled.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Quail embryos in the egg are exposed to 2450 MHz CW and pulsed radiation in a temperature controlled waveguide. The temperature of the embryo is carefully monitored with a thermistor and the heart rate determined using carbon impregnated polymer for the electrodes.

MAJOR FINDINGS AND PROPOSED COURSE: Reports in the literature show that the heart rate of perfused turtle and rat hearts is affected by exposure to microwave radiation. In our experiments embryonic quail hearts are exposed in vivo to 2450 MHz CW microwave radiation. No changes in heart rate have been observed that could not be explained by an increase in temperature on exposure to microwave radiation. Several exposure intensities ranging from less than 1 mW/cm² to 10 mW/cm² are used. These studies will be continued with pulsed microwave radiation of various intensities.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Since it has been reported that microwave radiation affects the heart rate of perfused hearts, it is important to determine if the same effect exist in vivo. In addition, if microwave radiation is found to affect the heart rate, the elucidation of the mechanism of action will be an important goal.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50026-01 LEB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Development of Closed System Electroacoustic Transducer for Noise Research		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Others;	Reginald O. Cook Teruzo Konishi Stanley Hutcheson	Acoustical Engineer Visiting Scientist Consultant LEB NIEHS LEB NIEHS UNC
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Environmental Biophysics		
SECTION Noise Effects Research		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina		
TOTAL MANYEARS: .25	PROFESSIONAL: .20	OTHER: .05
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline) For some time, the ability to perform experiments requiring the presentation of high level, wide band, minimum distortion sounds to the eardrum of guinea pigs has been unduly restricted by presently available small electroacoustical transducers suitable for earbar interface. As a result, high level sounds have had to be produced from loudspeakers mounted some distance from the ear (field stimulation). As a result, distortion, maximum achievable level and accuracy of measurement suffer. The availability of electroacoustic transducers operated into closed systems (earbar or ear canal) and capable of producing very high levels would allow one time acute exposures at levels sufficient to cause immediate auditory damage resulting in significant time savings. In addition, the pseudorandom noise which will serve as stimulus in some experiments, is characterized by a line spectrum. Prevention of distortion in line spectrums is particularly important. There have been reports in the high fidelity literature of earphone transducers having many of the desirable characteristics, so an attempt will be made to have such a device optimized for closed system applications.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Very sophisticated level, frequency and distortion tests of any electroacoustics transducer can be performed in our laboratory. Devices will be built to our specifications by others.

MAJOR FINDINGS AND PROPOSED COURSE: Small commercially available electro-acoustic transducers are optimized to operate into an acoustic impedance simulating that of the human ear canal and pinna, or about 6 cc. A change in this volume of as much as 10% causes significant frequency response and distortion changes. The acoustically compliant volume of conventional ear bars and guinea pig ears is about .5 cc. Mass and resistive loading are negligible. Manufacturers will be approached in an effort to get an optimized prototype built to our specifications.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Sound sources with which precise control (i.e., no distortion) of acoustic waveforms at the ear, across wide BW and amplitude can be produced. It is believed that current electroacoustic transducer technology can be exploited to achieve advances in noise exposure research.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50027-01 LEB												
PERIOD COVERED October 1, 1977 to September 30, 1978														
TITLE OF PROJECT (80 characters or less) Prenatal Exposure to High Noise Levels on Auditory Thresholds in Guinea Pigs														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT														
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 45%;">Reginald O. Cook</td> <td style="width: 20%;">Acoustical Engineer</td> <td style="width: 20%;">LEB NIEHS</td> </tr> <tr> <td>Others:</td> <td>Teruzo Konishi</td> <td>Visiting Scientist</td> <td>LEB NIEHS</td> </tr> <tr> <td></td> <td>Peter Nawrot</td> <td>Visiting Fellow</td> <td>LET NIEHS</td> </tr> </table>			PI:	Reginald O. Cook	Acoustical Engineer	LEB NIEHS	Others:	Teruzo Konishi	Visiting Scientist	LEB NIEHS		Peter Nawrot	Visiting Fellow	LET NIEHS
PI:	Reginald O. Cook	Acoustical Engineer	LEB NIEHS											
Others:	Teruzo Konishi	Visiting Scientist	LEB NIEHS											
	Peter Nawrot	Visiting Fellow	LET NIEHS											
COOPERATING UNITS (if any) Laboratory of Environmental Toxicology														
LAB/BRANCH Laboratory of Environmental Biophysics														
SECTION Noise Effects Research														
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina														
TOTAL MANYEARS: .8	PROFESSIONAL: .5	OTHER: .3												
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline)														
Pregnant guinea pigs will be exposed to tape recorded textile mill (weaving room) noise. The hearing level of offspring will be measured and compared with that of offspring of otherwise identical mothers. Study of the effects of high level sounds on fetuses <u>in utero</u> is important because: 1. Many women are moving into very noisy occupations, some as jet engine mechanics where whole body exposures exceed 120 dBA, 2. a period of enhanced susceptibility was recently demonstrated to occur in hamsters beginning at the point of final maturation of the ear, i.e., about 20 days post partum, 3. the equivalent period on humans and guinea pigs occurs near the end of the last trimester, 4. a Polish study recently found elevated hearing thresholds in guinea pigs exposed <u>in utero</u> to weaving room noise.														

PROJECT DESCRIPTION

METHODS EMPLOYED: Pregnant guinea pigs will be exposed to tape recorded weaving room noise in an acoustically shielded chamber, 8 hours per day, 5 days per week. The hearing level of offspring will be tested on at least two appropriate occasions by either the noninvasive sound evoked brain-stem response or N_1 action potential and by cochlear microphonic before sacrifice. Temporal bones (cochleas) will be saved for subsequent histological analysis, if indicated.

MAJOR FINDINGS AND PROPOSED COURSE: Because some uncertainty exists concerning the most efficient method for measuring hearing levels, a pilot study using 3 to 4 pregnant guinea pigs will be conducted to determine the most appropriate method. These animals will be exposed to higher noise levels (115 dBA) than proposed for the major study in order for better definition of any positive results, and animals will only be exposed during roughly the last trimester when deleterious effects are expected to occur, in order to save time.

Full term and last trimester exposures will be conducted during the full scale tests. It is anticipated that about 15-20 young will be tested on each of three occasions in these tests. The comparatively small number of test animals results from the hypothesis that damage occurs in utero and is not subject to progression. The experiment will be staged so that one pregnant female delivers per week, so that subsequent testing of offspring does not compromise the ability to pursue other experiments simultaneously.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Because of the rapid movement of females of childbearing age into occupations traditionally dominated by men, some of them very noisy, increasing concern has been voiced regarding possible general teratogenic and specific auditory risks to fetuses of mothers so exposed. One indication of this concern was the request made of the National Academy of Sciences by the Air Force that the former convene a scientific panel to assess that risk. The panel has not yet reported its findings.

Concurrently, in a 1977 report in Science, Bock and Sanders presented compelling evidence for the existence of a period of enhanced susceptibility to auditory system damage from noise insult in hamsters. The inception of this critical period corresponded to the beginning of structural maturation of the auditory system (approximately day 20 after birth in hamster) and continued for about 25-30 days. Because of the very short fetal stage in hamsters (18 days), the corresponding stage occurs prenatally in humans. Falk, Cook and Haseman have previously presented histological, and Price has reported electrophysiological evidence for enhanced auditory damage susceptibility of very young versus older guinea pigs. Recently Daniel, in a 1976 study in Poland, reported that the offspring of pregnant guinea pigs exposed to weaving mill noise (105 dBA) had higher threshold levels (poorer hearing) than the offspring of mothers not so exposed. Because of the comparatively long fetal period of guinea pigs (68 days), the period corresponding to auditory system structural maturation also occurs prenatally.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50028-01 LEB															
PERIOD COVERED October 1, 1977 to September 30, 1978																	
TITLE OF PROJECT (80 characters or less) The Effects of Noise and Drugs on the Electrochemistry of the Cochlea																	
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																	
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI:</td> <td style="width: 33%;">Teruzo Konishi</td> <td style="width: 33%;">Visiting Scientist</td> <td style="width: 10%;">LEB</td> <td style="width: 10%;">NIEHS</td> </tr> <tr> <td></td> <td>Alec N. Salt</td> <td>Visiting Fellow</td> <td>LEB</td> <td>NIEHS</td> </tr> <tr> <td>Others:</td> <td>Edward Lebetkin</td> <td>Biological Technician</td> <td>LEB</td> <td>NIEHS</td> </tr> </table>			PI:	Teruzo Konishi	Visiting Scientist	LEB	NIEHS		Alec N. Salt	Visiting Fellow	LEB	NIEHS	Others:	Edward Lebetkin	Biological Technician	LEB	NIEHS
PI:	Teruzo Konishi	Visiting Scientist	LEB	NIEHS													
	Alec N. Salt	Visiting Fellow	LEB	NIEHS													
Others:	Edward Lebetkin	Biological Technician	LEB	NIEHS													
COOPERATING UNITS (if any) None																	
LAB/BRANCH Laboratory of Environmental Biophysics																	
SECTION Noise Effects Research																	
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																	
TOTAL MANYEARS: 1.1	PROFESSIONAL: 0.8	OTHER: 0.3															
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																	
SUMMARY OF WORK (200 words or less - underline keywords) The objective of the project is to find physiological correlates to account for the loss of cochlear sensitivity during the initial stages of noise overexposure. The endocochlear potential, the tone-induced cochlear responses and the potassium activities of the endolymph and perilymph have been recorded in vivo from anesthetized guinea pigs. Changes in these parameters have been studied during repeated, discrete periods of acoustic overstimulation. When overstimulated with broad band white noise, although the cochlear microphonics and action potentials were suppressed the endolymph and perilymph potassium concentrations were maintained at their normal values. The endocochlear potential was found in most cases to be increased by acoustic overstimulation.																	

PROJECT DESCRIPTION

METHODS EMPLOYED: A double barreled, liquid membrane, ion-selective micro-electrode has been developed in order to make continuous recordings of the potassium activity of cochlear endolymph and perilymph independently of the electrical potential of the fluid. Electrodes with tip diameters between 2-4 μ and electrical impedances of approximately $2 \times 10^9 \Omega$ were used. Electrodes were inserted through the spiral ligament into scala media of the basal turn of the guinea pig cochlea and simultaneously recorded the endolymphatic potassium activity and the endocochlear potential (EP). Tone-induced cochlear potentials in response to 6 kHz tone bursts were recorded from differential electrodes positioned in the perilymphatic scalae.

Acoustic overstimulation was provided by broad band white noise, tones at 1 kHz or 8 kHz, or octave band noise centered at 400 Hz or 4 kHz at intensities ranging from 95 to 125 dBA.

MAJOR FINDINGS AND PROPOSED COURSE:

1. The potassium activity of cochlear endolymph measured by ion electrodes in vivo compared well with data obtained by the analysis of endolymph samples in vitro by conventional methods. The mean potassium activity recorded was 111.3 mM (S.D. 5.65). If the activity coefficient of potassium ions in endolymph is close to that of pure KCl solution, the estimated potassium concentration would be 153.4 mM. The insensitivity of the potassium electrode to changes in the EP (interference from the EP being a problem with earlier ion electrode studies of endolymph) was confirmed by inducing an anoxic state in a number of preparations. The resulting rapid decline of the EP did not induce any major changes in the endolymph potassium estimate.

2. The effect of high intensity noise on the tone-induced cochlear potentials, the EP and the endolymph and perilymph potassium concentrations has been studied. (i) When broad band white noise was presented at 95 dBA, the EP fell (this change often termed the negative summing potential) and then increased slightly during a 20 minute period of stimulation. On cessation of the noise, the EP increased by a similar amount to that at the onset. The sensitivity of the cochlea, as measured by the input/output functions of the cochlear microphonics and action potentials in response to 6 kHz tones, was unchanged by this exposure, as were the endolymph and scala tympani perilymph potassium concentrations. (ii) When preparations were exposed to white noise at 105 dBA, the EP fell at the onset as in the previous case but increased in value more rapidly during the period of stimulation and exceeded the pre-exposure value during the 20 minute exposure period. When exposure ceased the EP was further increased. This exposure produced a 4 dB loss of the cochlear microphonic response at the 100 μ V level whilst the endolymph and perilymph potassium concentrations were unchanged throughout the entire exposure. (iii) When white noise was presented at 115 dBA the EP fell but increased to become supernormal within the first minute of exposure. During the remaining period of stimulation, the EP was maintained above the pre-exposure level. Exposure at this intensity

for 20 minutes reduced the sensitivity of the cochlear microphonics by 22 dB at the 100 μ V level whilst the potassium activities of the endolymph and perilymph were unchanged. (iv) A study is presently being undertaken to ascertain whether the changes in the EP observed during noise exposure arise from the cochlear hair cells or as a result of vascular changes within stria vascularis. Preparations in which the hair cells have been damaged by the administration of ototoxic antibiotics will be subjected to similar noise exposures and the resulting changes of EP and endolymph potassium activity compared with the control group of data.

3. The effect of different types of noise on the cochlear responses has also been studied. Changes in response to tones at 1 kHz and 8 kHz and octave band noise centered at 400 Hz and 4 kHz were compared with those in response to white noise exposure. While a tone at 8 kHz or octave band noise centered at 4 kHz presented at 115 dB resulted in a fall in the EP followed by a rapid increase to above the pre-exposure level, a tone at 1 kHz or octave band noise centered at 400 Hz presented at the same intensity produced a slower increase in the EP. This suggests that the high frequency component of the noise is the main element responsible for the increase in EP in the basal turn during noise exposure.

We plan to expand the present noise exposure study in the following directions.

1. Noise exposure to the cochlea will be combined with the treatment of vaso-active or metabolically active drugs to ascertain whether the noise-induced changes of the cochlear potentials can be influenced by such treatment.

2. As the EP and potassium concentrations of endolymph and perilymph remain relatively unchanged during a noise exposure sufficient to reduce the cochlear microphonic output, it appears probable that the primary site of noise damage is at the hair cell level. With improvement of the ion electrode to obtain tip diameters of less than 1μ , it may be possible to pass electrodes through the organ of Corti to measure the electrochemical profile of the hair cells in normal and noise exposed preparations. This would provide valuable insight into the physiological basis of noise damage in the cochlea.

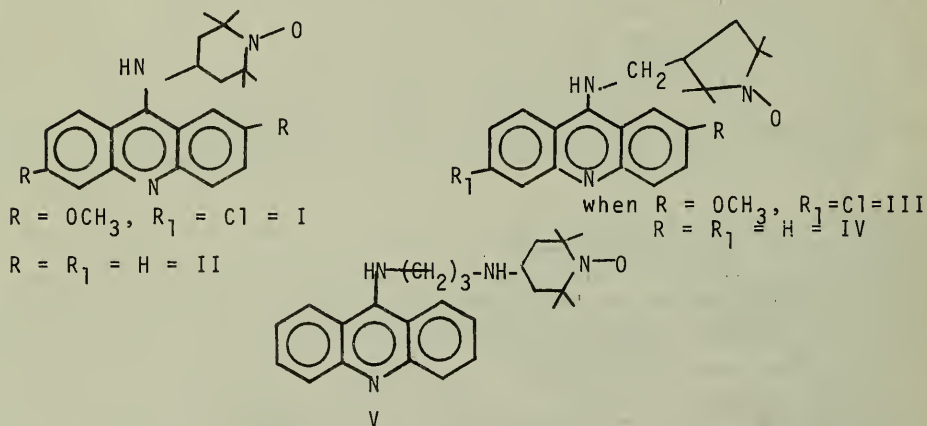
3. We also plan to examine the effect of impact noise on the physiological status of the cochlea in an attempt to quantify the difference between impact and continuous noise exposure in terms of the degree of auditory suppression produced.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Although it has been well documented that noise exposure results in a hearing deficit, the mechanism by which noise produces such a deficit has not yet been established. This project should provide valuable information concerning the basic mechanisms by which noise damages the cochlea.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50029-01 LEB										
PERIOD COVERED October 1, 1977 to September 30, 1978												
TITLE OF PROJECT (80 characters or less) Acridine Spin Labels as Probes for Nucleic Acids												
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT												
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI:</td> <td style="width: 33%;">Birandra K. Sinha</td> <td style="width: 33%;">Senior Staff Fellow</td> <td style="width: 10%;">LEB</td> <td style="width: 10%;">NIEHS</td> </tr> <tr> <td>Others:</td> <td>Colin F. Chignell</td> <td>Chief</td> <td>LEB</td> <td>NIEHS</td> </tr> </table>			PI:	Birandra K. Sinha	Senior Staff Fellow	LEB	NIEHS	Others:	Colin F. Chignell	Chief	LEB	NIEHS
PI:	Birandra K. Sinha	Senior Staff Fellow	LEB	NIEHS								
Others:	Colin F. Chignell	Chief	LEB	NIEHS								
COOPERATING UNITS (if any) NONE												
LAB/BRANCH Laboratory of Environmental Biophysics												
SECTION Molecular Biophysics												
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709												
TOTAL MANYEARS: 0.45	PROFESSIONAL: 0.45	OTHER: 0										
CHECK APPROPRIATE BOX(ES)												
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER												
☐ (a1) MINORS ☐ (a2) INTERVIEWS												
SUMMARY OF WORK (200 words or less - underline)												
The purpose of this project is to design spin label probes which can be used to study the binding of <u>mutagens to nucleic acids</u>. Since <u>acridines</u> are mutagenic and bind strongly to nucleic acids, we have designed and prepared acridine spin labels containing the stable nitroxide free radical. The binding of these acridine spin labels to nucleic acids has been studied by <u>electron spin resonance</u>. These spin label derivatives bind to nucleic acids by <u>intercalation</u> but are non base specific, i.e., they bind to both A-T and G-C base pairs in nucleic acids.												

PROJECT DESCRIPTION

METHODS EMPLOYED: The nature of the interaction between aminoacridines and nucleic acids is of considerable interest because of the mutagenicity of these compounds and their reported antitumor activity. The biological properties of the acridines are believed to arise either from their intercalation into DNA (or RNA) or from their association with phosphate groups on the outside of the double helix of nucleic acids. We have therefore synthesized aminoacridine spin labels I and V and studied their interaction with RNA and DNA by means of electron spin resonance and other spectroscopic techniques.



Acridine spin labels were synthesized from their corresponding 9-chloro-acridines and the respective spin labeled amines. Calf thymus DNA and calf liver RNA were purchased from a commercial source and used as received. Electron spin resonance (ESR) spectra were recorded with a Varian E-4 spectrometer operating at 9.5 GHz. Samples were introduced into the cavity in quartz micro flat cells. The temperature of the samples was maintained with a Varian variable temperature accessory and was measured with a Yellow Springs Instrument Company telethermometer. A Cary 15 spectrophotometer equipped with a water jacket was used for the T_m determinations. Sedimentation velocity measurements were made with a Beckman Spinco Model E analytical ultracentrifuge.

MAJOR FINDINGS AND PROPOSED COURSE: The ESR spectrum of acridine spin label I bound to DNA was broad and asymmetric with a maximal hyperfine splitting ($2T_m$) of 58.7 G. Neither heat denaturation of the DNA nor the addition of salt (0.1 M NaCl) altered the $2T_m$ value of bound label I (Table I). The $2T_m$ value of acridine spin label II bound to DNA was 55.5 G. The addition of 0.1 M NaCl to the DNA-II complex did not significantly alter the ESR spectrum of the probe (Table I).

TABLE 1
THE MAXIMAL HYPERFINE SPLITTINGS ($2T_m$) OF ACRIDINE SPIN LABELS
BOUND TO CALF THYMUS DNA

Sample	NaCl (0.1 M)	$2T_m$ (G)
DNA + I	-	58.7
DNA (heat denatured) + I	-	59.0
DNA + I	+	59.0
DNA + II	-	55.5
DNA + II	+	55.0
DNA + III	-	66.5
DNA + III	+	67.0
DNA + IV	-	57.0
DNA + IV	+	57.0

In the absence of spin label, DNA was found to have a sedimentation coefficient of 11.2 S while on the addition of spin label I, the sedimentation coefficient decreased to 8.92S. This observation suggests that label I is indeed intercalated into the DNA. The effect of the acridine spin labels on the melting temperature (T_m) of DNA was studied by means of absorption spectroscopy and electron spin resonance. The T_m value for DNA alone was found to be 66.5° as determined by the increase in the optical density of the solution at 260 nm. In the presence of spin label I, the melting temperature of DNA was raised to 70°. It was also found possible to measure the T_m value for complexes between the spin labels I and II and DNA by monitoring the concentration of free spin label in solution as function of temperature. The T_m value of a complex between DNA and I was found to be 69° by this technique. The complex between DNA and II exhibited T_m values of 76° by the spectrophotometric assay and 78° by the ESR method.

The binding parameters for the interaction between I and II and DNA were determined by titrating a fixed amount of nucleic acid with increments of spin label and measuring the concentration of free probe by ESR. Both spin labels exhibited at least two kinds of binding sites on DNA (Table 2). At low ionic strength, it is known that binding occurs both by intercalation and an electrostatic interaction between the dye and the nucleic acid. The addition of a high concentration of salt is reported to abolish binding due to the electrostatic process. It will be seen from Table 2 that in the presence of 0.1 M NaCl the affinity of both spin labels for DNA is drastically reduced. From the ESR spectra of complexes between labels I and II and DNA, it is apparent that the piperidine ring that bears the nitroxide has greater mobility in the complex between DNA and II. This suggests that there is a fundamental difference in the way that these two spin labels interact with DNA. This difference is also reflected in the melting temperatures of complexes between I and II

and DNA with spin label II producing a greater increase in the T_m value. Molecular models of labels I and II demonstrate a pronounced steric effect of methoxyl group in the 2 position which forces the acridine ring into a conformation in which it is perpendicular to the flat piperidine ring. This observation may explain the differences in the mobility of the nitroxide group of labels I and II bound to DNA. It is also possible that the conformational

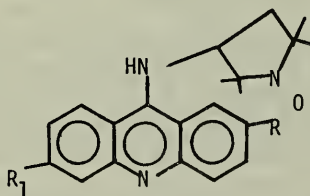
TABLE 2

Sample	N_1	Binding Parameters		
		K_1 ($M \times 10^{-5}$)	N_2	K_2 ($M \times 10^{-5}$)
I	0.150	120.0	2.11	0.74
I + 0.1 M NaCl	0.127	16.1	1.88	0.079
II	0.186	93.0	0.18	0.27
II + 0.1 M NaCl	0.163	19.7	0.22	0.22

differences between the acridine labels may explain why II is more effective in raising the melting temperature of DNA.

The ESR spectrum of label I bound to calf liver RNA indicated that the spin label had a higher degree of mobility than when bound to DNA. It is likely that the calf liver RNA exists in a single stranded form. It therefore appears possible that these spin labels may be useful in detecting double helical and single stranded regions in nucleic acids.

For further studies two more acridine spin labels (VI + VII) are being prepared in which chain length is decreased. The binding of these spin labeled acridines to nucleic acids and to synthetic polymers will be studied in detail. In addition, binding of repressor and melting proteins and of histones to nucleic acids is also planned.



When $R = OCH_3$, $R_1 = Cl = VI$

$R = R_1 = H = VII$

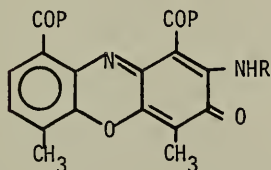
SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Acridines are of interest for two reasons; firstly, because of their biological and pharmacological effects and secondly, because they may be useful tools for probing the conformation of nucleic acids in biologically important complexes such as chromatin, DNA-repressor proteins, etc. In both areas, it is expected that these acridines spin labels will provide useful information, not only on the pharmacological and toxicological effects of acridines in biological systems, but also in the area of genetranscription.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50030-01 LEB						
PERIOD COVERED October 1, 1977 to September 30, 1978								
TITLE OF PROJECT (80 characters or less) Spin Label Actinomycin-D and Adriamycin as Base Specific Probes for Nucleic Acids								
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Birandra K. Sinha</td> <td style="width: 33%;">Senior Staff Fellow</td> <td style="width: 33%;">LEB NIEHS</td> </tr> <tr> <td>Others: Colin F. Chignell</td> <td>Chief</td> <td>LEB NIEHS</td> </tr> </table>			PI: Birandra K. Sinha	Senior Staff Fellow	LEB NIEHS	Others: Colin F. Chignell	Chief	LEB NIEHS
PI: Birandra K. Sinha	Senior Staff Fellow	LEB NIEHS						
Others: Colin F. Chignell	Chief	LEB NIEHS						
COOPERATING UNITS (if any) None								
LAB/BRANCH Laboratory of Environmental Biophysics								
SECTION Molecular Biophysics								
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709								
TOTAL MANYEARS: 0.45	PROFESSIONAL: 0.45	OTHER: 0						
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS								
SUMMARY OF WORK (200 words or less - underline)								
The purpose of this project is to prepare base specific spin label probes to study binding of chemical carcinogens to nucleic acids. For this purpose, we plan to prepare spin labeled actinomycin-D to probe G-C-regions in nucleic acids. In addition, we also plan to prepare spin labeled adriamycin for binding studies of the A-T regions of the nucleic acids.								

PROJECT DESCRIPTION

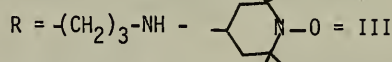
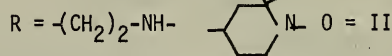
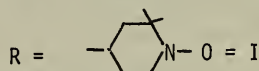
METHODS EMPLOYED: The purpose of the present work is to develop base specific probes for studying the binding of chemical carcinogens and mutagens to nucleic acids. Since, the binding of these agents to nucleic acids is base specific, it is of considerable interest to design and prepare base specific spin label probes to evaluate the mechanism of binding of carcinogens to nucleic acids and characterize those binding sites responsible for carcinogenesis.

Actinomycin-D is one of the few antitumor agents possessing curative effects against Wilm's tumor and gestational choriocarcinoma. The mechanism of action of Actinomycin-D appears to result from intercalation of flat ring systems into G-C base pairs of double-standard DNA which leads to an inhibition of protein synthesis. The base specificity of Actinomycin-D in binding to DNA, has prompted us to design and prepare labels I-III to probe the binding of certain carcinogens to the G-C base pairs.



Where P = $\overbrace{\text{Thr-D-Val-Pro-Sar-Me-Val}}$

When R = H = Actinomycin-D



Adriamycin, a synthetic analog of Daunomycin, is a potent antitumor drug. The activities of Daunomycin and adriamycin have been related to their ability to specifically interact with A-T base pairs in nucleic acids. We plan to prepare spin labeled analogs of adriamycin and Daunomycin to probe A-T binding sites in nucleic acids.

MAJOR FINDINGS AND PROPOSED COURSE: This project is in the initial stages, efforts so far have been concerned with preparation of the probes. Binding studies with these probes and DNA will be carried out using electron spin resonance (ESR).

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Since the binding of chemical carcinogens to nucleic acids is base specific, it is hoped that by preparing and utilizing base specific spin labeled probes, we would be able to define and understand the interaction of carcinogen with nucleic acids at a molecular level. In addition, these probes will also aid in understanding binding of histones and repressor proteins to nucleic acids.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50031-01 LEB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Molecular Mechanisms of Mast Cell Degranulation		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Mary J. Ortner Other: Colin F. Chignell Staff Fellow Chief LEB NIEHS LEB NIEHS		
COOPERATING UNITS (if any) NONE		
LAB/BRANCH Laboratory of Environmental Biophysics		
SECTION Molecular Biophysics		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) We have used <u>fluorescent probes</u> , <u>light scattering</u> and <u>circular dichroism</u> to study the molecular interactions of <u>compound 48/80</u> . We have shown that pretreatment of <u>rat peritoneal cells</u> and <u>mastocytoma cells</u> by 48/80 increases the binding of both <u>1-anilinonaphthalene-8-sulfonate</u> and <u>Rhodamine-labeled 48/80 (R-48/80)</u> . The binding of <u>12-anthroyl-stearic acid</u> was not affected. The emission maximum of R-48/80 did not change after binding to mastocytoma cells, whereas that of <u>12-anthroyl-stearic acid</u> showed a blue shift indicating binding in a region of low polarity relative to the R-48/80. <u>Fluorescence microscopy</u> of <u>mast cells</u> labeled with R-48/80 showed it bound to the <u>plasma membrane</u> at low concentrations and to <u>intracellular granules</u> at high concentrations. There was no <u>patching</u> or <u>capping</u> . <u>Light scattering</u> by intact cells and cell membranes was increased by both 48/80 and heat-treatment. Protein depleted liposomes were not affected. <u>Ultraviolet circular dichroism</u> studies of mastocytoma cell membranes showed that 48/80 and heat-treatment however, did not significantly alter the ultrastructure of the membrane proteins. It is concluded that 48/80 binds to a receptor which is basically different from that of the fatty acids and which may be a protein.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Rat mast cells were isolated according to well established methods. Mastocytoma cells were an established cell line grown in CDF-1 mice. Red blood cell ghosts were prepared according to the method of Dodge, et al. Fluorescence and light scattering were studied using Perkin-Elmer SPF-44A spectrophotofluorimeter and circular dichroism was studied using a Jasco J40A automatic recording polarimeter.

MAJOR FINDINGS AND PROPOSED COURSE:

1. Fluorescent probe studies. We have studied the effect of compound 48/80 on the fluorescence of membrane-bound 1-anilinonaphthalene-8-sulfonate (ANS). Mastocytoma cells incorporate ANS (as indicated by increasing fluorescence intensity) rapidly at first, and then more slowly for a duration of 15-20 minutes. Compound 48/80 increased significantly the incorporation of ANS whether it was added before or after the chromophore. This effect of 48/80 was dose dependent. Normal peritoneal and plural cells extracted from rats also incorporate ANS in a manner similar to the mastocytoma cells. The fluorescence of ANS in mastocytoma cells was quenched by spin labeled fatty acids, which indicates that the fluorescent probe and the fatty acids are binding in close proximity (4-6 Å) on the cell membrane.

Compound 48/80 was labeled covalently with the fluorescent moiety, rhodamine (R-48/80) and studied in mastocytoma cells using the differential corrected spectra attachments of the spectrophotofluorimeter. Mastocytoma cells pretreated with unlabeled 48/80 show increased incorporation of R-48/80 as demonstrated by increased fluorescence. This indicates that 48/80 pretreatment can also increase its own binding as well as that of ANS. The emission maximum (Em) of R-48/80 (excited at 350 nm) did not change when it was incorporated into the mastocytoma cell membranes or into the intact cells. When the fatty acids are incorporated into biological membranes, the Em of the attached chromophore always shows a blue-shift. This indicates that the fatty acids are in a more hydrophobic environment relative to physiological saline. Since R-48/80 does not change its Em on binding to cell membranes, it is concluded that its environment in cell membranes is similar in polarity to that of the physiological saline. We have also found that pretreatment of mastocytoma cells by 48/80 does not increase the binding of fluorescent fatty acids. This in contrast to its effect on ANS and R-48/80.

We have explored the feasibility of photographing the binding sites of 48/80 on mastocytoma and mast cells. Cells were treated with various quantities of R-48/80 and the excess drug removed. Using fluorescence microscopy we have shown that R-48/80 binds to the cells in a manner which can be seen and photographed. Mast cells and mastocytoma cells incorporate R-48/80 at high concentrations (10-1000/ml) to the extent that it enters the cells and binds to anionic sites within the cell. In mast cells these are primarily the mast cell granule and in mastocytoma cells, the nucleus. At low concentrations R-48/80 binds to the plasma membrane in a uniform manner, and does not exhibit

patching or capping. This pattern is similar for mast cells and mastocytoma cells.

2. Light scattering and circular dichroism studies

Rayleigh scatter was measured by locking the spectrophotofluorometer monochrometers together and scanning identical wavelengths between 200-600 nm. Three different preparations from mastocytoma cells were studied: intact cells, cell membranes and membrane-derived liposomes. All of these preparations scattered light, however, the pattern was different in each case due to the absorption of light of various wavelengths by nucleic acids and proteins. The addition of 48/80 to the intact cells and also to cell membranes resulted in a significant increase in light scattering at all wavelengths where absorption did not interfere. This effect was also seen in normal peritoneal cells and in purified mast cells. Rayleigh scatter from protein-depleted liposomes, however, was not affected by compound 48/80. All cells which were heat treated (45°C, 10 min) showed an increase in light scatter which was similar to that seen with 48/80 treatment. Furthermore, heat-treated cells showed an increase in light scattering by 48/80 which was significantly larger than that of normal cells. None of the above effects were seen with the inactive 48/80 monomer p-methoxy-N-methylphenethylamine.

To determine the role of protein on light scatter, mastocytoma membrane-derived lipids were sonicated in the presence of bovine serum albumin (BSA). Although control liposomes did not show increased Rayleigh scatter with 48/80, those formed in the presence of BSA showed a slight increase after 48/80 treatment. To investigate further the effect of 48/80 on protein, ultraviolet circular dichroism was used to determine whether 48/80 could affect protein ultrastructure. The results indicated that 48/80 did not disturb the configuration of BSA in solution or in liposomes. When mastocytoma cell membranes were examined, 48/80 did cause a change in the absorption pattern of the membrane proteins. This effect, however, proved to be due to 48/80-induced precipitation of nucleic acids in the membrane preparation. Heat treatment did not affect the CD absorption of the membrane or whole cell preparation.

We have continued the work of the previous year by investigating further the action of compound 48/80 on a molecular basis. We have found that 48/80 affects both ANS and R-48/80 in a manner similar to the local anesthetics. That is, the fluorescence intensity is increased in a dose-related manner by pretreatment with 48/80. This may be due to a change in the charge density of the membrane and/or an increase in the number of receptor sites available. Since 48/80 pretreatment did not affect the fluorescence emitted by anthroyl derivatives of stearic acids, it is reasoned that its binding site(s) is of a character which is basically different from that of stearic acids. However, since the fluorescence of ANS and Rhodamine-48/80 is quenched by spin-labeled fatty acids, their binding sites are probably in close proximity. Through fluorescence microscopy we have seen that this site(s) appears to be distributed evenly on the plasma membrane. Since R-48/80 does not change its fluorescence emission maximum after membrane binding, this site(s) is further presumed to be relatively apolar and close to the surface. Because the effect

of 48/80 on membrane Rayleigh scatter was similar to that induced by heat-denaturation of membrane proteins and dependent on the presence of proteins, it is proposed that 48/80 binds to the membrane proteins and that its action may include their partial denaturation.

We are currently studying the effect of 48/80 on spin-labeled probes such as 5-doxy1 and 12-doxy1 stearic acid. Although we have not found any change in the environments of these probes, we plan to study others including spin-labeled phospholipids and fluorescent labeled phospholipids, cholesterol and 16-anthroy1 stearic acid. Since these probes bind to different regions of the cell membrane, we may be able to detect changes in their environment caused by 48/80. We have also synthesized spin labeled 48/80 and are continuing our previous years work on the study of its binding site(s).

Recently, it has been postulated that mast cell degranulation generates free radicals in a manner similar to that seen during phagocytosis. In preliminary studies we have found that two different free radical scavengers ("spin traps") have an inhibitory action on 48/80-induced mast cell degranulation. This would imply that a free radical may constitute an important or perhaps necessary intermediate for mast cell degranulation. We are currently searching for direct evidence of free radical generation by 48/80 in mast cells. The identification of such a product would definitely aid in a better understanding of the action of 48/80 and the phenomenon of mast cell degranulation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In order to understand better the underlying mechanisms behind noncytotoxic histamine secretion, we have studied the artificial histamine liberator, compound 48/80. This drug has been labeled successfully with the fluorescent probe rhodamine and its action studied in cell membranes. In addition, other spectroscopic probes have been used to study membranes from mast cell mastocytoma cells and other cell types. With the aid of these tools, we are trying to identify the binding site(s) of 48/80 in order to elucidate the molecular mechanism of histamine secretion from mast cells. The inflammatory reaction mediated by mast cells is a major contributing factor to the adverse symptoms of photo-allergic and phototoxic dermatitis. In addition it may also exacerbate the conjunctivitis and pulmonary distress caused by certain xenobiotics in the atmosphere. Our studies into the precise molecular mechanism of histamine secretion may lead to the development of a safe method of control over these environmentally related adverse reactions.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50032-01 LEB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Binding of Copper to Human Serum Albumin		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: P. Mohanakrishnan Others: Colin F. Chignell Visiting Fellow Chief LEB NIEHS LEB NIEHS		
COOPERATING UNITS (if any) NONE		
LAB/BRANCH Laboratory of Environmental Biophysics		
SECTION Molecular Biophysics		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline) The interaction of <u>copper</u> with <u>human serum albumin</u> is being examined with the aid of a number of spectroscopic techniques. In addition to albumin itself, binding studies are also being made with the N-terminal tripeptide of albumin and with larger peptide fragments isolated from enzymatic digestion of albumin. In these studies the amino acids that function as chelation sites for copper will be identified. This work will provide a molecular basis for the interaction between copper and human serum albumin in both normal and diseased states (Wilson's disease, rheumatoid arthritis and Bechterew's disease).		

PROJECT DESCRIPTION

METHODS EMPLOYED:

1. Tripeptides with the same amino acid sequences as in N-terminal end of human serum albumin (HSA) will be synthesized.
2. Cleavage and isolation of N-terminal fragments of HSA will be carried out.
3. Spectroscopic techniques such as the nuclear magnetic resonance (NMR), electron spin resonance (ESR), fluorescence and circular dichroism will be used to monitor the binding of Cu^{2+} .

MAJOR FINDINGS AND PROPOSED COURSE: N-methylamide of the tripeptide L-Asp-L-Ala-L-His will be synthesized. Studies of the binding of Cu^{2+} with the tripeptide will be carried out using spectroscopic techniques.

Cu^{2+} is known to enhance the relaxation rates of protons at the binding site, thereby causing line broadening in the NMR spectra. On the other hand, Ni^{2+} causes only shifts of the NMR peaks. The relaxation and shift data have been used in obtaining geometry information of molecules in solution bound to metal ions. Studies will be carried out to obtain any possible information about the Cu^{2+} binding site using both proton and carbon-13 NMR. These studies will be extended to human serum albumin and its N-terminal fragments.

Both proton and carbon-13 NMR spectra of proteins consist of numerous overlapping peaks and hence NMR spectral assignment for proteins are extremely difficult. The proton and carbon-13 NMR spectra of simple peptides are of considerable assistance in simplification of spectral assignments. Once the assignment of at least some peaks has been made, it is possible to study the conformational transitions by observing the changes taking place in the spectra. Studies of conformational changes induced Cu^{2+} binding will be carried out using both NMR and CD.

Lanthanide metal ions are known to fluoresce more when bound to ligands than in the free state. Decreases in fluorescence intensity of a HSA-bound lanthanide metal ion such as terbium or europium when displaced by Cu^{2+} , will be useful in finding the binding constants. Energy transfer between HSA-bound lanthanide metal ions and intrinsic fluorophores such as tryptophan and tryosine can be of assistance in obtaining geometric information about these residues relative to the Cu^{2+} binding site.

The NMR, ESR and fluorescence techniques will be used for studying the molecular dynamics of HSA in solution. The kinetics of Cu^{2+} -HSA binding will also be carried out by the stopped-flow technique.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The waste effluents from several energy-related processes including oil shale production and coal gasification contain high concentrations of heavy metal ions including Cu^{2+} . The binding of heavy metals by human serum albumin has important toxicological implications since this protein can act as both a

carrier and a "sink" for these ligands. Thus it is of paramount importance to understand the factors which govern the interaction between metal ions and serum albumin.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50033-01 LEB												
PERIOD COVERED October 1, 1977 to September 30, 1978														
TITLE OF PROJECT (80 characters or less) Do Nitroaromatic Free Radicals Covalently Bind to Macromolecules?														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%; vertical-align: top;">PI:</td> <td style="width: 35%; vertical-align: top;">Ronald P. Mason</td> <td style="width: 35%; vertical-align: top;">Research Chemist</td> <td style="width: 15%; vertical-align: top;">LEB NIEHS</td> </tr> <tr> <td style="vertical-align: top;">Others:</td> <td style="vertical-align: top;">B. Kalyanaraman</td> <td style="vertical-align: top;">Visiting Fellow</td> <td style="vertical-align: top;">LEB NIEHS</td> </tr> <tr> <td></td> <td style="vertical-align: top;">J.L. Holtzman</td> <td style="vertical-align: top;">Pharmacologist</td> <td style="vertical-align: top;">U. of Minn.</td> </tr> </table>			PI:	Ronald P. Mason	Research Chemist	LEB NIEHS	Others:	B. Kalyanaraman	Visiting Fellow	LEB NIEHS		J.L. Holtzman	Pharmacologist	U. of Minn.
PI:	Ronald P. Mason	Research Chemist	LEB NIEHS											
Others:	B. Kalyanaraman	Visiting Fellow	LEB NIEHS											
	J.L. Holtzman	Pharmacologist	U. of Minn.											
COOPERATING UNITS (if any) Department of Pharmacology, University of Minnesota														
LAB/BRANCH Laboratory of Environmental Biophysics														
SECTION Molecular Biophysics														
INSTITUTE AND LOCATION NIEHS, Research Triangle Park, North Carolina														
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0												
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less on a separate sheet) We have proposed that nitroaromatic anion free radicals (RNO₂⁻) covalently bind with glutathione, protein or with other tissue macromolecules, $\text{RNO}_2^- + \text{P} \longrightarrow \text{RNO}_2 - \text{P}$ where P represents a macromolecule or glutathione. In most studies on the covalent binding of reductive intermediates of nitro compounds to protein it is usually assumed that the hydroxylamine metabolite is the metabolite that covalently binds. Yet there is only very circumstantial evidence to support this assumption. Since as much as 14% of a reductive intermediate of some nitrofurans becomes covalently bound to protein, it should be possible to observe spectroscopically the covalent binding of the anion radicals, if this intermediate is indeed responsible for binding. Because reduced glutathione depletion is thought to be important in some drug toxicities, it is important to determine if the anion radical can directly bind to glutathione. In addition, L-cysteine or GSH decreased the covalent binding of one nitrofurans carcinogen by 99.9% without affecting the microsomal nitroreductase activity. Just recently the binding of a reductive metabolite(s) of two nitrofurans to DNA and RNA has been reported.														

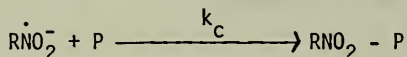
PROJECT DESCRIPTION

METHODS EMPLOYED: Electron spin resonance will be utilized to detect steady-state levels of nitrocarcinogen free radicals.

MAJOR FINDINGS AND PROPOSED COURSE: We wish to examine the effect of added glutathione, protein, DNA and RNA on the nitrofur anion radical kinetics. The steady-state concentration of anion radical as determined by an ESR spectrometer, will be altered by protein concentration if the radical binds to protein. Covalent binding of RNO_2^- to glutathione, protein, or other macromolecules, P, will compete with the disproportionation of the radical as a decay mechanism according to the following rate expression,

$$\frac{d[\text{RNO}_2^-]}{dt} = k_1 [\text{E}] [\text{RNO}_2] - 2k_d [\text{RNO}_2^-]^2 - k_c [\text{RNO}_2^-] [\text{P}],$$

where k_c is the rate of covalent binding by the anion radical to protein, and $k_1 [\text{E}] [\text{RNO}_2]$ is the rate of enzymatic radical formation.



The steady state condition yields

$$[\text{RNO}_2^-]_{ss} = \frac{-k_c [\text{P}] + \sqrt{k_c^2 [\text{P}]^2 + 8k_d k_1 [\text{E}] [\text{RNO}_2]}}{4k_d}$$

Any reversible association between P and E or RNO_2 will be reflected in k_1 . If k_1 is unchanged by increasing P, but the steady state radical concentration is reduced by increasing P, then RNO_2^- is probably covalently binding to P.

Preliminary work in Dr. Holtzman's laboratory indicates that even at 100 mM GSH does not affect the steady-state concentration of a nitrofur anion radical in microsomal incubations. Published work indicates that the GSH does not affect microsomal nitroreductase activity, but can decrease protein binding by 99.9% at 0.4 mM. These early results indicate that RNO_2^- is not the reductive metabolite(s) that covalently binds to protein.

In conclusion, although RNO_2^- does not appear to react with GSH or protein, it may react with DNA or RNA. In any case, other work has shown that RNO_2^- is an obligate first intermediate of microsomal nitroreductase, and its formation is therefore necessary to the formation of an even more reactive intermediate(s) which does bind to GSH, protein, DNA and RNA.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The nitro carcinogens, five of which are available from NCI's chemical repository, are thought to require reductive activation before they can initiate tumor formation. The binding of a reductive intermediate to tissue macromolecules

is thought particularly important in this regard. The investigation of any reactions between RNO_2^- and GSH or macromolecules will contribute to our understanding of the mechanism of nitro activation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50034-01 LEB						
PERIOD COVERED October 1, 1977 to September 30, 1978								
TITLE OF PROJECT (80 characters or less) The Mechanism of the Oxygen-Insensitive Activation of 4-Nitroquinoline-1 oxide								
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%;"> <tr> <td style="width: 33%;">PI: Ronald P. Mason</td> <td style="width: 33%;">Research Chemist</td> <td style="width: 33%;">LEB NIEHS</td> </tr> <tr> <td>Others: B. Kalyanaraman</td> <td>Visiting Fellow</td> <td>LEB NIEHS</td> </tr> </table>			PI: Ronald P. Mason	Research Chemist	LEB NIEHS	Others: B. Kalyanaraman	Visiting Fellow	LEB NIEHS
PI: Ronald P. Mason	Research Chemist	LEB NIEHS						
Others: B. Kalyanaraman	Visiting Fellow	LEB NIEHS						
COOPERATING UNITS (if any) NONE								
LAB/BRANCH Laboratory of Environmental Biophysics								
SECTION Molecular Biophysics								
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709								
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0						
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS								
SUMMARY OF WORK (200 words or less - underline) With one exception all mammalian nitroreductases are markedly inhibited by oxygen. Previous investigations have found that the <u>oxygen-insensitive nitroreductase</u> of rat liver supernatant is DT diaphorase. The reduction of 4-nitroquinoline-1-oxide (4-NQO) by supernatant was inhibited by oxygen only 10%, whereas dicoumarol inhibited 94%. The microsomal reduction of 4-NQO is oxygen sensitive as is characteristic of the usual nitroreductases. Several lines of evidence suggest that the oxygen inhibition of nitroreductases is the result of the air oxidation of the first reduction intermediate, the nitroaromatic anion <u>free radical</u> (RNO_2^-). $\text{RNO}_2 + \text{O}_2 \longrightarrow \text{RNO}_2^- + \text{O}_2^-$ In view of the known rapid rate of the air oxidation of nitro anion free radicals ($k > 2.5 \times 10^5 \text{ M}^{-1} \text{ sec}^{-1}$), this <u>oxygen-insensitive nitroreductase</u> must not form the RNO_2^- or, at the very least, the radical must remain bound to the DT diaphorase and not be accessible to O_2 . Presumably in this case, the first reduction intermediate formed is the corresponding nitroso compound, the two-electron reduction product.								

PROJECT DESCRIPTION

METHODS EMPLOYED: Electron spin resonance will be used to search for the anion free radical metabolite of 4-nitroquinoline-1-oxide (4-NQO). Oxygen uptake and the rate of NADPH oxidation will be determined using standard techniques. The adrenochrome assay used for superoxide is a modification of the method of Misra and Fridovich.

MAJOR FINDINGS AND PROPOSED COURSE: If the mechanism of microsomal nitro-reduction of 4-NQO is like that of other oxygen-sensitive nitroreductases, then anaerobic incubations of microsomes, 4-NQO and NADPH should form the anion free radical, which should be detectable by electron spin resonance. Under aerobic conditions the free radical should not be detectable, but 4-NQO should stimulate O_2 uptake, superoxide formation, and NADPH oxidation by the microsomal incubation via the cyclic formation of the nitro anion free radical.

In contrast, we expect that incubations containing an equal amount of supernatant nitroreductase activity will not form the 4-NQO anion free radical, as determined by electron spin resonance. Again, in contrast to the microsomal incubations, the supernatant incubation should not consume more oxygen or oxidize more NADPH in the presence of 4-NQO.

Lastly, supernatant incubations should not form more superoxide in the presence of 4-NQO, whereas microsomal incubations are expected to generate more superoxide. Since the nitro compound-catalyzed oxygen consumption, superoxide formation and NADPH oxidation are thought to be due to the rapid air oxidation of RNO_2 , these results would support the electron spin resonance results concerning the formation of the 4-NQO anion radical in the microsomal and supernatant incubations.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Either the nitroso or hydroxylamine metabolites of 4-NQO are thought to be the proximate carcinogen of 4-NQO. The rapid cellular reduction of 4-NQO in air is unique among nitro compounds and is thought responsible for its potent carcinogenicity relative to that of other nitro compounds and its greater toxicity and mutagenicity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50035-01 LEB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Interpretation of the ESR Spectra of Spin-labeled Membranes		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Ronald P. Mason Research Chemist LEB NIEHS		
COOPERATING UNITS (if any) NONE		
LAB/BRANCH Laboratory of Environmental Biophysics		
SECTION Molecular Biophysics		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline k McConnell, et al. have developed an <u>S</u> parameter method of spectral interpretation almost universally used in the analysis of spin-labeled membrane spectra. Hubbell and McConnell have emphasized that for their S formalism to be valid, one must have $\text{Trace } T = \text{Trace } T'$, where T is the nitrogen hyperfine tensor in the principal axis system and T' has the components T' and T'. This requirement follows directly from the rotational invariance of the trace of a tensor. In the work of Hubbell, et al variations in a_N , the isotropic nitrogen hyperfine splitting, have been interpreted as changes in the polarity of the local environment of the nitroxide group, and a correction has been used to adjust the measured S for the polar-hydrophobic effect. This polarity profile within the hydrocarbon region of the bilayer has been attributed to electrostatic interactions between the nitroxide dipole and the dipolar regions of the bilayer. Although the polarity profile does probably exist in many cases, a breakdown of assumptions made in the derivation of the S parameter formalism could cause an artifactual polarity change which is actually a violation of the necessary condition that $\text{Trace } T = \text{Trace } T'$.		

PROJECT DESCRIPTION

METHODS EMPLOYED: All ESR spectra of microsomal membranes will be recorded with a Varian E-109 spectrometer equipped with a Varian TM₁₁₀ cavity. Temperatures other than 22°C will be achieved with a Varian temperature controller using the variable temperature flat cell and dewar designed for the TM₁₁₀ cavity. Both the microwave power and the modulation amplitude will be chosen as one-tenth of the values necessary to maximize the signal of the center line.

MAJOR FINDINGS AND PROPOSED COURSE: The isotropic nitrogen hyperfine splitting, a_N , has been found to be independent of temperature in a variety of organic solvents. Even rigid limit spectra taken at liquid nitrogen temperature have the same a_N as spectra at room temperature or above. The parameter a_N for spin-labeled membranes has been reported to be a function of temperature. The result has been thought to represent a real change in the polarity profile of the membrane. We intend to measure a_N for spin-labeled egg lecithin as a function of temperature in the vicinity of room temperature and at liquid nitrogen temperatures where a_N can be determined independently of motional effects. We expect that a_N at liquid nitrogen temperatures will be temperature independent and different from the apparent value determined by the extrapolation of a_N values near room temperature, which will be determined using the Hubbell-McConnell Formalism.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many workers in the biological sciences are using spin labeling to investigate phenomena which are thought or have been shown to be related to membrane fluidity, such as cancer, muscular dystrophy, complement-induced membrane damage and the mechanism of action of lytic compounds. Changes in the apparent a_N are often described in these studies. In many of these studies an increase in a_N is accompanied by an increase in S . Although rigorous simulations of spectra have shown that changes in the rotational rate of the nitroxide about its symmetry axis of rotation can cause simultaneous changes in a_N and S of the kind reported in the literature, experimental proof is needed of artifactual changes in a_N .

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50036-01 LEB								
PERIOD COVERED October 1, 1977 to September 30, 1978										
TITLE OF PROJECT (80 characters or less) A Search for Free Radical Metabolites of Polycyclic Hydrocarbons										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT										
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI:</td> <td style="width: 33%;">B. Kalyanaraman</td> <td style="width: 33%;">Visiting Fellow</td> <td style="width: 15%;">LEB NIEHS</td> </tr> <tr> <td>OTHERS:</td> <td>Ronald P. Mason</td> <td>Research Chemist</td> <td>LEB NIEHS</td> </tr> </table>			PI:	B. Kalyanaraman	Visiting Fellow	LEB NIEHS	OTHERS:	Ronald P. Mason	Research Chemist	LEB NIEHS
PI:	B. Kalyanaraman	Visiting Fellow	LEB NIEHS							
OTHERS:	Ronald P. Mason	Research Chemist	LEB NIEHS							
COOPERATING UNITS (if any) NONE										
LAB/BRANCH Laboratory of Environmental Biophysics										
SECTION Molecular Biophysics										
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina										
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0								
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS										
SUMMARY OF WORK (200 words or less - underline) We propose to use <u>electron spin resonance</u> to search for <u>free radical metabolites</u> of <u>polycyclic hydrocarbons</u> and <u>dibenzodioxins</u> . Free radicals of electron donor molecules are formed in the peroxidase reaction according to the following mechanism. $\begin{aligned} \text{Peroxidase} + \text{H}_2\text{O}_2 &\longrightarrow \text{Compound I} + \text{H}_2\text{O} \\ \text{Compound I} + \text{R} &\longrightarrow \text{Compound II} + \text{R}^{\bullet+} \\ \text{Compound II} + \text{R} &\longrightarrow \text{Peroxidase} + \text{R}^{\bullet+} \end{aligned}$ Many compounds are known to undergo such a <u>one-electron oxidation</u> in the presence of a variety of <u>peroxidases</u> and hydrogen peroxide. <u>Electron spin resonance spectra</u> have been obtained of the free radical metabolites of hydroquinone and N-hydroxy-2-acetylaminofluorene and many biological compounds. This class of enzymes appears to have little substrate specificity, any electron donor with a sufficiently low oxidation potential should be metabolized to a free radical. In addition to lactoperoxidase and myeloperoxidase, hemotin, methemoglobin and cytochrome P-420 all have peroxidase activity.										

PROJECT DESCRIPTION

METHODS EMPLOYED: Electron spin resonance will be utilized to detect free radical intermediates. Difference absorption spectra will be used to follow the formation and decay of compound I and II.

MAJOR FINDINGS AND PROPOSED COURSE: Ten $\mu\text{l/ml}$ of 30% H_2O_2 will be added to an incubation mixture containing horseradish or lactoperoxidase and the tetra-sulfonic acid derivative of pyrene in acetate buffer (0.2 M pH 4.6). The incubate will then be aspirated through a stainless steel needle into an aqueous flat cell mounted in the microwave cavity of the E-109 spectrometer. The ESR spectra at room temperature will be immediately determined. The exploratory investigations will be performed at acidic pH because the peroxidases are usually most active and the stability of the cation radical is expected to be greater under acidic conditions.

The cation free radicals of aromatic hydrocarbons and dibenzodioxins have been previously made by chemical methods and their hyperfine patterns and g-values characterized, therefore the identification of any free radical metabolite should be straightforward. If a spectrum is found, the free radical's dependence on enzyme concentration, hydrogen peroxide concentration, and pH will be determined. In addition, the effect of peroxidase inhibitors such as cyanide and alternate substrates such as ascorbate will be investigated. Similar experiments with other polycyclic hydrocarbons and various dibenzodioxins are also planned.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The metabolic activation of aromatic hydrocarbons by one-electron oxidation has been proposed to be of importance in the mechanism of tumor initiation by many workers, but not one has present evidence of the enzymatic formation of the proposed cation free radicals. The formation of the analogous cation free radical of 2,3,7,8-tetrachlorodibenzo-p-dioxin has been proposed as the oxidative metabolite which binds to rat liver microsomes.

UNIVERSITY OF WASHINGTON

TITLE: "Effects of Microwave Radiation on the Nervous System"

CONTRACTOR'S PROJECT DIRECTORS: Richard H. Lovely, Ph.D.
Arthur W. Guy, Ph.D.

PROJECT OFFICER (NIEHS): Donald I. McRee, Ph.D., Research Physicist
Laboratory of Environmental Biophysics

DATE INITIATED: June 20, 1976

CURRENT FUNDING LEVEL: \$118,919

PROJECT DESCRIPTION

OBJECTIVE: The objective of this project is to determine the effects of long-term, low-level, exposure to 918 and 2450 MHz microwave radiation on the central nervous system and behavior.

METHODS EMPLOYED: Long-Evans male rats, 80-90 days of age will be exposed to 915 and 2450 MHz circularly polarized microwave radiation at a power density level such that the specified absorption rate will be equivalent to that produced in a 5 mW/cm² plane wave field. The exposure will be 8 hours a day, 7 days a week for 4 months. Two month post-irradiation evaluations will be performed. Rats will also be exposed to 2450 MHz plane wave microwave field at a power density of 500 μ W/cm² using the same schedule as that stated above. The following indicators of biological effects will be made:

1. Biochemistry - Serum content for sodium, calcium, phosphorus, PTH, SH groups and cholinesterase activity of the blood and 17-ketosteroids in urine will be measured.
2. Behavior - Animals will be evaluated for free-operant and free-repertoire behavior. Animals will be observed in the irradiation chambers and their specific orientation to the field, sleep, grooming, sniffing, rearing, drinking, eating, etc., will be recorded.
3. Electroencephalography - EEG recordings will be made on the animals following the four month irradiation period.

In addition to the above endpoints, some behavioral teratology, cataract evaluation, and longevity data will be obtained.

MAJOR FINDINGS AND PROPOSED COURSE: Eight pregnant female rats were exposed 20 hrs/day during 19 days of gestation to 5 mW/cm², 918-MHz CW microwave fields without apparent effect upon their health or subsequent deliveries. Additionally, no discernible physical or physiological deficits characterized the dam's offspring at birth nor throughout development. However, when tested as adults, these offspring demonstrated a significant behavioral deficit during acquisition of a conditioned avoidance response. Evidence suggests disruption of the normal pituitary-adrenal axis response to stress affected performance in this task. These findings demonstrate the value of adult behavioral protocols as a supplement to more standard morphological measures employed to assess

teratogenic effects of energy related pollutants.

Eight female rats were exposed to $500 \mu\text{W}/\text{cm}^2$ ($0.5 \text{ mW}/\text{cm}^2$) for 7 hours/day, 7 days a week for 3 months in individual anechoic chambers. All animals were exposed from above in individual chambers with a field closely simulating a plane wave. Monthly assessments of urinary ketosteroids failed to reveal any difference between exposed and sham-exposed control rats. Blood cholinesterase and sulfa-hydral (glutathione) activity revealed significant initial decrements in the exposed animals relative to the controls followed subsequently by no differences between groups at the end of months 2 and 3. Evaluation of serum electrolytes, CO_2 , and BUN at the end of the three-month exposure protocol revealed significant differences in Na^+ , K^+ , CO_2 , and ion gap. After a one-month recovery period, the evaluation of all these parameters was repeated and found that the exposed group still differed significantly with respect to Na^+ and ion gap but appeared to be "recovering". In the context of our other observations of these animals, we are inclined to hazard a preliminary diagnosis of aldosteronism--an interesting and feasible possibility given the vertical exposure protocol, (i.e., a regional hot spot in the adrenal zona glomerulosa).

Behavioral testing of shock sensitivity monthly, and terminal tests of open field activity and shuttlebox avoidance responding also revealed significant differences between the groups at the end of three month's exposure.

Eight female rats will be exposed to 2450 MHz circularly polarized CW microwave radiation at a power density level such that the specified absorption rate will be equivalent to that produced in a $5 \text{ mW}/\text{cm}^2$ plane wave field. The exposures will be for 8 hours per day for 4 months. Effects on biochemistry, behavior and EEG will be measured. Pregnant rats will also be exposed and behavioral teratology will be assessed in the rats exposed in utero after they mature.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The Institute has, as part of its mission, the responsibility for studying the biological effects of physical factors in the environment. The potential health effects of low-level, long-term microwave exposure is not now known. With the increase of microwave radiation in the environment, it is important to determine the effects of microwave exposure. The most often reported effect in the literature is on the central nervous system and behavior. It is important that the Institute study long-term, low-level, effects since very little research at these conditions is presently being done in the United States.

PUBLICATION

Johnson, R.B., Mizumore, S., and Lovely, R.H.: Adult behavioral deficit in rats exposed prenatally to 918 MHz microwaves. Proceedings 17th Hanford Biology Symposium. (In press).

UNIVERSITY OF UTAH

TITLE: "Effects of Microwave Radiation on the Nervous System"

CONTRACTOR'S PROJECT DIRECTOR: Om P. Gandhi, Ph.D.

PROJECT OFFICER (NIEHS): Donald I. McRee, Ph.D., Research Physicist
Laboratory of Environmental Biophysics

DATE CONTRACT INITIATED: June 28, 1976

CURRENT ANNUAL LEVEL: \$78,884

PROJECT DESCRIPTION

OBJECTIVE: The objective of this project is to determine the effects of long-term, low-level exposure to 915 and 2450 MHz microwave radiation on the central nervous system and behavior.

METHODS EMPLOYED: Long-Evans male rats will be exposed to a CW plane wave microwave field at frequencies of 915 and 2450 MHz and power density of 5 mW/cm^2 . The animals will be exposed for 8 hours a day, 5 days a week for 16 weeks. An eight week recovery period will follow the exposure period. The following measurements will be made:

1. Biochemistry - Biochemical analysis of the cholinesterase activity of the blood, the SH group in the blood, and ketosteriods in the urine will be measured.
2. Behavior - Rodent activity will be measured using a rodent activity wheel. Water and food intake will be measured. Rats will be trained to lever press for food reward on a FR-DRL multiple schedule. Total response and reinforcement for each schedule component as well as response during time out between each component will be recorded. Interresponse times on each schedule component will be recorded.
3. EEG - EEG will be recorded biweekly using epidural removable stainless steel electrodes. These recordings will be made while the microwave radiation is turned off.

MAJOR FINDINGS AND PROPOSED COURSE: The exposure of Long-Evans rats to 918 MHz CW microwave radiation has been completed. The exposure was for 5 days per week for 16 weeks to a power density of 5 mW/cm^2 in a plane wave field. An assessment of "innate" locomotor behavior using a running wheel and activity platform were made. No changes were measured in the exposed group when compared with the sham controls. An assessment of rat performance on a multiple fixed ratio-differential reinforcement of low response rates (FR-DRL) reward schedule was also performed. Preliminary analysis of the data indicates a drop in the responses per second on the FR component of the schedule in the exposed animals.

Hematological and serum chemistry and urine chemistry has been carried out on the animals. Only one parameter, total free sulfhydryl groups, showed a difference (increase) between the irradiated and sham irradiated animal groups. This

difference was indicated during the first week of irradiation and thereafter returned to baseline levels.

Exposure of rats to 2450 MHz CW microwave radiation at 5 mW/cm power density will be carried out in the next part of the experiment. The same biological tests will be run as those noted above. In addition, the effects of both 915 MHz and 2450 MHz on EEG will be investigated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The biological effects of long-term, low-level microwave exposure on the central nervous system and behavior is not known. Presently, only a small amount of research is being performed to evaluate the health hazards of this type of radiation. This research will contribute to the determination of whether these fields do or do not constitute a health hazard. This project directly applies to the mission of the Institute in evaluating the health effects of physical factors in the environment.

UNIVERSITY OF CALIFORNIA AT LOS ANGELES (UCLA)

TITLE: "Effects of 60 Hz Fields on the Mammalian Central Nervous System"

CONTRACTOR'S PROJECT DIRECTOR: W. Ross Adey, M.D.

PROJECT OFFICER (NIEHS): Donald I. McRee, Ph.D., Research Physicist
Laboratory of Environmental Biophysics

DATE CONTRACT INITIATED: October 1, 1975

CURRENT ANNUAL LEVEL: \$60,000

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this research are to determine the effects of 60 Hz fields from high voltage transmission lines on the central nervous system of mammals.

METHODS EMPLOYED: Environmental field conditions similar to those found in the vicinity of high voltage transmission lines will be simulated. These field gradients will range from 1 to 2000 volts/meter. The effects of these fields on the biological rhythms in rats will be evaluated. The effects on the neuroendocrine mechanism in rats will be examined by using 17-hydroxysteroids, calcium, phosphorus, vanilmandelic acid and other urinary indicators of bio-rhythmicity. Changes in the neurochemistry of brain tissue of cats will be studied. These studies will include the measurement of both Ca^{2+} and amino acid effluxes from the cerebral cortex of the cat.

MAJOR FINDINGS AND PROPOSED COURSE: Exposure of rats to 60 Hz electric fields at 50, 500, and 1000 volts/meter (V/m) have been completed. Exposures at 500 and 1000 volts/meter and a complete sham exposure have been duplicated with an interval of approximately six months between these paired tests. Preliminary results indicate a possible increase in movement in the first 7 days of exposure in the 500 V/m exposed group and the first 3 days of the 1000 V/m group with a return toward sham levels after these periods. Urine output was higher for animals in 500 and 1000 V/m throughout the epoch of field exposures. This difference in urine output did not parallel differentials in body weight. Fluid intake increased by approximately 20 percent for all groups over the entire experiment with no evidence of differences between exposed and sham exposed groups. An increase in weight gain occurred in the 50 V/m and 500 V/m groups, and a decrease in the 1000 V/m group. Average weight of wet adrenal tissue was higher (57.8 mg) for 500 V/m group and for 1000 V/m (50.8) than in the no-field group (43.1 mg). No differences were detected in averaged wet thyroid tissue weights. No changes in any of the examined hematological parameters were measured.

Fractionated urine specimen will be examined in the rats for levels of calcium, potassium, and phosphorous, using atomic absorption techniques. Excretion patterns of these ions exhibit strong circadian periodicities and will be analyzed so as to evaluate any such changes.

A series of 5 monkeys trained in operant procedures (DRL schedules of reinforcement) already shown to be sensitive to 7 and 75 Hz. ELF fields will be tested in 60 Hz fields. It is planned to test their sensitivity to fields up to 2000 V/m.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The electric power industry is planning to construct high voltage transmission lines with voltages up to 1200 kV. In the vicinity of the lines, high electric field gradients will be present. The health effects of these fields are presently unknown. This research will contribute to the determination of whether these electric fields do or do not constitute a health hazard. This project contributes to the mission of the Institute in evaluating the health effects of physical factors in the environment.

PUBLICATIONS

Adey, W.R.: Models of membranes of cerebral cells as substrates for information storage. BioSystems 8: 163-178, 1977.

Adey, W.R.: The sensorium and the modulation of cerebral states: tonic environmental influences on limbic and related systems. Ann. New York Acad. Sci., 290: 396-420, 1977.

Bawin, S.M., Sheppard, A. and Adey, W.R.: Mechanisms of weak electromagnetic field coupling in brain tissue. In Taylor, L.S. and Cheung, A.Y. (Ed.): The Physical Basis of Electromagnetic Interactions with Biological Systems, University of Maryland. Office of Naval Research and Bureau of Radiological Health, Food and Drug Administration, pp. 75-89, 1977.

Bawin, S.M., Sheppard, A. and Adey, W.R.: Models of long-range order in cerebral macromolecules: effects of ELF, VHF and UHF fields on calcium binding. URSI International Symposium on the Biological Effects of Electromagnetics Waves, Airlie, Virginia, 1977.

Bawin, S.M., Sheppard, A. and Adey, W.R.: Possible mechanisms of weak electromagnetic field coupling in brain tissue. Bioelectrochemistry and Bioenergetics, 1978.

UNIVERSITY OF MIAMI

TITLE: "Protracted Noise Exposure and Cardiovascular Function"

CONTRACTOR'S PROJECT DIRECTOR: Ernest A. Petterson, Ph.D.

PROJECT OFFICER (NIEHS): Teruzo Konishi, M.D., Ph.D., Visiting Scientist
Laboratory of Environmental Biophysics

DATE CONTRACT INITIATED: January 1977

CURRENT ANNUAL LEVEL: \$40,000

PROJECT DESCRIPTION

OBJECTIVES: Because of the serious and pervasive nature of cardiovascular diseases, including hypertension, the relationship between noise and cardiovascular function has received particular attention. The objectives of this contract are to specify (1) the extent to which protracted noise exposure produces cardiovascular adjustments, (2) the dynamics of those adjustments, and (3) the nature and mechanisms of adaptation to noise. Semi-restrained rhesus monkeys are used for the work.

METHODS EMPLOYED: Rhesus monkeys will be exposed for a period of up to one year to noise conditions resembling those presently and potentially existing in the community and work place. Their cardiovascular responses will be compared with those of a group of rhesus monkeys maintained in relatively quiet conditions during the same period. Evidence for acoustic injuries and other organic pathology will be sought. During the course of the study, the general health condition will be monitored by various clinical tests.

MAJOR FINDINGS AND PROPOSED COURSE: Various kinds of everyday stimuli have been recorded with a professional quality cassette recorder. The majority of equipment purchases and renovation of the experimental quarters have been completed. The development of softwares of a computer is now underway. The project will be approached as outlined above.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The work will provide scientific information which is vital to further refine our concepts of how long-term exposure to contemporary noise levels affects the cardiovascular system in humans.

LABORATORY OF ENVIRONMENTAL MUTAGENESIS

THE LABORATORY OF ENVIRONMENTAL MUTAGENESIS

Summary Statement

The Laboratory of Environmental Mutagenesis carries out research concerning the role of environmental mutagens in human health. This year the Laboratory was split into two Laboratories, the main body of workers remaining with the LEM and a smaller group of workers forming the new Laboratory of Biochemical Genetics. Although the LEM continues to take the major responsibility in the broad area of environmental mutagenesis, its increasing size dictates the need for even further splitting in order to create groups which can work more effectively because of their smaller sizes and in order to allow their scientific leaders time to devote to scientific rather than only to administrative matters. Since the discipline of environmental mutagenesis is both new and very broad in technical and scientific scope (ranging from viral to mammalian genetics and from metabolic activation to theoretical population genetics), considerable further growth and continued recruitment of scientific leadership is anticipated in the next few years.

The recognition over the last decade of the potential role of environmental chemicals in the etiology of much modern disease has stimulated considerable interest and growth in the area of environmental mutagenesis. There has been remarkable public awareness of and concern for the importance of protecting the human genome from genetic damage that would constitute a legacy for many generations into the future. More immediately, the recognition that even simple microbial mutation assays may be efficient screens to pick out potential human mutagens and carcinogens had led to explosive growth in the development and application of this particular methodology. The establishment and development of the Laboratory was and continues to be a serious attempt to respond to these needs. Although growth has been steady, it has occurred with continuous re-evaluation of direction and emphasis.

The scope of research that falls within the domain of environmental mutagenesis is quite large. Geneticists within the Laboratory are working with such diverse organisms as phages, bacteria, yeasts, the bread mold Neurospora, the fruit fly Drosophila, and the mouse, with occasional contributions from yet other organisms. On the biochemical side are investigations ranging from the basic structure and function of relatively simple molecules, through the pharmacological behavior of multi-enzyme complexes which process environmental chemicals, to the analysis of the structure and detailed function of major macromolecules such as proteins and nucleic acids. Some of the Laboratory programs are expected to have an immediate impact on environmental health science (for instance, the development of improved mutagenicity screens), while others are expected to have their impact in the more distant future, being targeted towards the development of an understanding of basic genetical mechanisms or fundamental base line measurements (for instance, investigations into the enzymes which act upon premutational lesions in DNA, or into the frequency of deleterious alleles in populations and the forces which maintain them).

Because of this diversity, and because of the administrative factors already mentioned, the Laboratory will soon divide into two units. One of these, to be called the Laboratory of Molecular Genetics, will be primarily concerned with molecular and microbial approaches to environmental mutagenesis. The other, the Laboratory of Animal Genetics, will concentrate on problems involving the genetics of whole animals and animal populations. Close cooperation among all three genetical Laboratories is anticipated, however, and this will extend to collaborative research projects.

MOLECULAR GENETICS

This is a new group whose primary focus is upon the diversity of molecular mechanisms which underlie the mutation process, starting with the induction of lesions in DNA and proceeding through various mutation avoidance and mutation fixation processes. A clear understanding of the induction and metabolism of DNA lesions is fundamental to a confident and successful program of environmental mutagenesis containment; note, for instance, that virtually all of the present screening systems, together with our capabilities and uncertainties as to how to interpret the results they generate, are based upon the molecular genetics of the past three decades. The molecular genetics group is approaching mechanistic problems utilizing both genetic and biochemical techniques.

Employing sophisticated viral chromosome mechanics to determine specific base pair substitution mutational pathways, the mechanism of "heat mutagenesis" is being investigated. Heat is an ever-present mutagen and a potentially important component of spontaneous mutation, and the mutagenic mechanisms it triggers are also likely to be enhanced by certain chemicals as well (particularly those capable of deaminating cytosine, or breaking purine glycosidic bonds); it has been estimated that the average human cell suffers more than 100 such mutations per day, most but not all of which are subsequently reverse by crucial repair and mutation avoidance systems. A part of the story of heat mutagenesis also involves the ability of the DNA synthesizing system to overcome the deleterious effects of unrepaired lesions, an ability which would be of considerable public health significance if we could learn how to manipulate it to advantage.

A number of the specific determinants of fidelity during both normal DNA synthesis and that perturbed by environmental chemicals are being investigated, with special emphasis on the ability of DNA polymerases, and other enzymes which work together with these polymerases, to overcome the effects of DNA damage. In related studies, attempts are being made to understand why some genes are much more mutable than others, with the answer now developing that site-specific mutability depends strongly upon certain specific base-pair sequences.

In another series of investigations, certain repair systems which sacrifice fidelity in order to repair DNA damage under "last chance" circumstances are being studied. An attempt is underway to discover all of the genes determining such error-prone repair, and to characterize their individual roles.

The detailed biochemistry of the DNA replication process is being investigated in vitro by combining purified components required for DNA synthesis and

determining the nature of their particular interactions. The study of inhibitors of DNA replication, and of mutants which have become resistant to such inhibitors, is steadily building up a more complete picture of the dynamics of DNA replication from which an overall understanding of the process of fidelity will emerge.

MICROBIAL GENETICS

The development and deployment of rapid, economical and sensitive microbial screens for mutagenicity constitute important advances in environmental health sciences. These assays are technically much more efficient than most classical toxicological test systems, but are by no means proven fully capable of detecting all potential human mutagens. Besides being simple, they are also very general assays, in the sense that in the aggregate they can detect many classes of mutational damage. They also frequently include important pharmacological components from mammalian tissue, and thus in some circumstances mimic the human body itself. The microbial geneticists are providing research needed to support and further this important component of environmental mutagenesis containment.

Considering the large number of chemicals that will have to be screened in the coming decade - the number runs into many thousands, and eventually into tens of thousands - it is crucial to organize the screening process so that it is known to be reliable and so that the vast amount of resulting data can be efficiently assimilated. To these ends this group has mounted a collaborative project, the Environmental Mutagen Test Development Program (EMTDP), involving not only diverse geneticists, but also chemists, statisticians and data-handling experts both within and outside of the Institute. Those within the Institute concentrate on the development of suitable methods, and on conducting testing by means of an extensive contracting system involving laboratories throughout the country (some being commercial, others based in universities). Through these contracts hundreds of chemicals will be screened each year using a blind experimental design. Some four contracts are now in effect, together with additional related contracts to cover the handling of the test chemicals and the resulting test data, and the statistical analysis of test results. Estimates of laboratory-to-laboratory variability and other sources of error will provide a more sound foundation upon which to base future and larger screens. This program is also expected to provide critical information bearing upon the correlation between the mutagenic and the carcinogenic activities of chemicals. Of course, the program is also expected to directly reveal mutagenic activity in numerous previously untested chemicals, thus providing an immediate as well as a future impact upon the environmental health problem.

A further development in the testing program will be the testing of selected chemicals in higher organisms. Such testing is much more expensive, much slower, and often reduced in sensitivity, but the results are often held to be more relevant to human health because of the closer relation to humans of test organisms such as fruit flies (and mice, for that matter). A *Drosophila*

geneticist has been recruited to manage the next testing phase, and will arrive in the upcoming fiscal year.

Another important component of the EMTDP will be the exploration of structure/function relations among mutagenic chemicals. To the extent that such relationships can be reliably established, even if only moderately correlational, they can be used in turn to assist in the prioritizing of chemicals for future testing. Such prioritizing is important because the number of chemicals to be tested presently far exceeds our capacity to test them.

A variety of systems development work is also being conducted by this group, either within the LEM or by contract with outside laboratories. Much effort has been expended this year to improve the metabolic activating systems which are so important in converting microbial test systems into pseudo-mammals, thus sharply reducing the great costs of whole-mammal screens involving large numbers of chemicals. Techniques such as improved activating enzyme induction, injection of the microbial system into the rodent blood system, the use of organ perfusion methods, direct use of rat hepatocytes *in vitro*, and even the innate ability of eukaryotic microbes to activate chemicals under certain conditions, have all been explored. A qualitatively new assay system has been developed which employs as its genetic end-point the generation and induced segregation of duplications. A simplified *Neurospora* test is also under continuing development; the effects of incorporating permeability and repair-defective mutations into these strains has been explored, as well as simplified mutation scoring procedures.

The microbial group has also investigated the possible mutagenicity in a variety of systems of certain chemicals of particular importance. Among the chemicals showing genetic activity are chlorinated hydrocarbons, the aflatoxins produced by fungal action on foods such as nuts, a number of chemotherapeutic agents, and a toxin produced by the penicillium fungus which grows in certain cheeses. Furthermore, the mutations produced in a number of previous tests employing the *Neurospora* forward-mutation system are being characterized as to type of lesion through a contract with Miles Laboratory, in order to determine the mutagen specificity of such chemicals with a view to eventual risk quantification.

Another test system development is also being conducted by contract, in this case with the University of British Columbia. At present there is no facile microbial eukaryotic system to screen environmental chemicals for their abilities to induce chromosome loss through meiotic nondisjunction. Such a genetic endpoint is an important component of human genetic disease, with typically very drastic effects upon the zygote. The system under development employs special genetically marked strains of *Neurospora*, and should be vastly less expensive to employ with large numbers of chemicals than are the higher eukaryotic systems. It should be noted that this system may also be able to detect genetic activity in a class of chemicals which escape detection by more conventional systems.

BIOCHEMICAL GENETICS

Our understanding of basic point mutational processes in higher eukaryotes, and especially in mammalian systems, depends primarily upon the analysis of mutant gene products. Although there are now strong hints that direct DNA sequencing, and both genetic and physical methods for genetic fine-scale mapping, are likely to become possible with higher eukaryotes in the next few years, it is still necessary at the present time to characterize mutants by parameters such as altered protein kinetic properties and sequence analyses. Two groups worked in this general area over the past year.

A newly formed group has established a sophisticated laboratory for protein sequencing. Their present work centers on the primary structure of mammalian lactate dehydrogenases, with particular attention to the sperm-specific form of this enzyme, LDH-X. Ongoing work in another NEIHS Laboratory is directed towards the development of a mutational assay whose end point would be an alteration in the pattern of LDH-X enzymatic activity as brought about by amino acid substitutions. A knowledge of the amino acid sequence of the parental form of the enzyme may therefore become important in the near future, to be followed by sequence analyses of mutant forms of the enzyme at a later date. Furthermore, since a part of the analysis of mutation will involve immunological variation, the sequence analysis will be extended to determining the antigenicity of various fragments of the protein.

Another group has been concentrating on methods to purify and characterize numerous other enzymes coded by the target genes which might be employed in new mutational assays in mammals. A thorough description of both the wild type and mutant enzymes is necessary in order to determine whether variation detected in the mutation experiments is in fact due to altered protein sequences, as expected from a true mutational response, or to altered protein post-translational modifications, which might reflect a nonmutational, epigenetic alteration such as occurs frequently during the course of differentiation. This group is therefore developing a broad description of the biochemical genetics of the mouse, one which will allow the facile analysis of new mutants and provide data upon which to found the development of more highly refined biochemical mutation assays. Many mouse enzymes have already been purified by rapid affinity chromatography, and then characterized by a wide array of enzymological and physical methods.

MAMMALIAN GENETICS

This program includes several simultaneous approaches to the study of mutagenesis in mammals. At the present time assays for point mutations in the mouse, while crucial for the risk estimation procedures which will be needed in the near future, are extremely expensive and tedious, and are rather insensitive because of inherent difficulties in screening large numbers of treated genes. The most promising way out of this dilemma would be to develop a recessive lethal screening system, perhaps along the lines so highly developed in the fruit fly; such a system may be capable of screening hundreds of genes simultaneously. Two approaches to this end are already under way. One, operating

via a contract with the Jackson Laboratory, seeks to obtain "balancer" chromosomes containing large inversions; these permit substantial fractions of particular mutagen-treated chromosomes to be maintained intact (not subject to recombination) over successive generations, until such chromosomes can be tested for their accumulation of recessive lethal mutations. Another (in-house) approach involves the same genetic end point, but need not wait for the slow accumulation of stocks of balancer chromosomes over many years; it examines the genome of the descendants of a treated mouse, but is complicated by the mixing effects of genetic recombination.

Translocations are also a well recognized component of human genetic disease. Both the transmissibility and the developmental effects of mutagen-induced translocations are therefore being determined in the mouse; and it is already clear that different heritable translocations produce characteristic but different transmission frequencies and developmental defects.

The study of mutations such as recessive lethals and heritable translocations in the mouse is a relatively costly and time-consuming business in the intact mammal, but direct knowledge of their induction and properties is essential to a comprehensive analysis of environmental mutagenesis, and especially to mammalian risk analysis. However, mutation can also be studied, far more rapidly and less expensively, in cultured mammalian cells. The induction of mutation in such cells is therefore being examined from several perspectives. Forward-mutation selection assays based on recognized human inborn errors of metabolism are proving to be useful both as test systems and as tools to better understand the process of mutagenesis itself in such cells. Correlations are being sought in several different types of mammalian cells between the induction of point mutations and of chromosome aberrations, and these responses are also being associated with the actual binding of the active form of the mutagen to cellular DNA.

A recently developed and thus far very promising indicator of exposure to mutagens is the induction of sister-chromatid exchanges (SCE). Unlike ordinary meiotic recombination, or even rare mitotic recombination, SCE involves exchanges between identical daughter chromosomes, rather than between homologous (maternal/paternal) but genetically quite distinct chromosomes; and SCE is usually believed to reflect a basic recombination-like postreplication repair process acting upon unexcised DNA lesions. Although the detailed mechanism of SCE is far from understood, SCE induction is now known to correlate very well with the more usual mutational end points (such as point mutations or chromosome aberrations). The ability of various mutagens and teratogens to induce SCEs is being studied in newly developed *in vivo* systems, rather than in the usual *in vitro* system; the development of a technique that will allow a test compound to be administered to an intact animal is often to be highly preferred since it is expected to give a pharmacologically more realistic and relevant response.

A number of important base-line studies are also being conducted by this group. These consist of comparisons of different types of somatic cells as indicators of chromosomal damage scored cytogenetically; and comparisons of different mouse

strains for their sensitivities to mutagens. It is highly likely that humans will exhibit considerable diversity in their responses to mutagens, for reasons having to do with basic enzymatic differences among individuals; whereas typical mouse test strains are highly inbred, and may not be at all representative of the variation which exists in nature.

POPULATION GENETICS

Future regulatory decisions about permissible human exposures to environmental mutagens will have to rest not only upon estimates of the specific mutagenicities of numerous chemicals, but also upon a detailed knowledge of the genetic structure of the exposed population: both sorts of information are required in order to estimate the magnitudes of induced increases in the frequencies of deleterious genes. There remain many areas of ignorance concerning the genetic basis of differences among individuals (in general health, in development, and in both physical and mental abilities), and concerning the degree to which these differences are related to mutation rates. The population genetics group is primarily concerned with gathering base-line information about genetic variation in natural populations. The classical model system for studies of this sort, one which is vastly less expensive and better understood than are humans themselves, is Drosophila. Most of the group's present research effort is devoted to the study of heritable enzyme deficiencies, which are close analogues of a variety of human biochemical genetic diseases. Extensive surveys of such variation are required in order to provide starting points from which well founded models can be obtained describing the dynamics by which such mutants arise and disappear in populations.

Another project involves measuring the induction of mutationally modified or abolished enzymes by low-level chronic gamma irradiation; it should be noted that such chronic exposure is much more characteristic of real environmental exposures, whether they be radiations or chemicals, than are the acute exposures usually delivered in laboratory experiments. One important result from this study has been the observation that such chronic-irradiation mutation rates are not very different from acute-irradiation mutation rates.

One area of genetics which has been largely ignored in the past by workers in environmental mutagenesis is cytoplasmic genetics; nevertheless, there are important genetic determinants in the cytoplasm. Genetic variation has therefore been explored in the mitochondrial DNAs among diverse species of Drosophila, and has been observed to be substantial.

While recombination is usually considered to be a normal and innocuous genetic process, it is known to be closely tied to the mutational response in several ways: components of the recombination process are often also components of certain DNA repair processes; mutagens typically increase rates of all types of recombination; and certain types of recombination promote the conversion of relatively less deleterious mutational heterozygosity to mutational homozygosity. A part of this group is therefore working to characterize genetically determined variation in recombination rates, both in terms of laboratory experimentation and by the development of theoretical models.

ENVIRONMENTAL MONITORING

While some chemicals can be brought directly into the laboratory for testing, others may not be known to exist in the environment, and their mutagenic activities can only be detected by direct environmental monitoring. A contract with the Brookhaven National Laboratory is supporting the field testing of a Tradescantia mutation assay. This plant shows extraordinary sensitivity to airborne mutagens as expressed in somatic-mutation end points, and is possibly the best system available for monitoring the mutagenicity of air pollutants. Early results indicate positive mutagenic responses in several industrial cities. This system therefore offers a technology to fill an important gap in environmental monitoring.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60004-05 LEM										
PERIOD COVERED October 1, 1977 to September 30, 1978												
TITLE OF PROJECT (80 characters or less) Development of Simple Mutagen Test Systems in <u>Neurospora crassa</u>												
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI:</td> <td style="width: 33%;">T. Ong</td> <td style="width: 33%;">Geneticist</td> <td style="width: 10%;">LEM</td> <td style="width: 10%;">NIEHS</td> </tr> <tr> <td>Other:</td> <td>J. Guthrie</td> <td>Microbiologist</td> <td>LEM</td> <td>NIEHS</td> </tr> </table>			PI:	T. Ong	Geneticist	LEM	NIEHS	Other:	J. Guthrie	Microbiologist	LEM	NIEHS
PI:	T. Ong	Geneticist	LEM	NIEHS								
Other:	J. Guthrie	Microbiologist	LEM	NIEHS								
COOPERATING UNITS (if any) None												
LAB/BRANCH Laboratory of Environmental Mutagenesis												
SECTION 												
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709												
TOTAL MANYEARS: 0.3	PROFESSIONAL: 0.2	OTHER: 0.1										
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (a1) MINORS ☐ (a2) INTERVIEWS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER												
SUMMARY OF WORK (200 words or less - underline keywords) N23 and N24, selected from hundreds of ad-3 mutants, have been used as testers for the spot, plate, and suspension tests in <u>Neurospora crassa</u>. These two testers are highly sensitive to <u>mutagens</u> and are revertible by specific groups of chemicals. N23 can be reverted from adenine-dependence to adenine-independence by agents which cause <u>base-pair</u> substitutions, whereas N24 can be reverted by <u>frameshift</u> mutagens. Spot, plate, and suspension tests using testers N23 and N24 are satisfactory substitutes for the ad-3 forward mutation system for screening the mutagenic activity of environmental agents and chemical carcinogens in <u>N. crassa</u>. Further studies will be conducted to validate these test systems and to determine whether the sensitivities of these testers to chemicals can be enhanced by incorporation of <u>UV sensitivity</u> and <u>Osmotic</u> markers into these tester strains.												

PROJECT DESCRIPTION

METHODS EMPLOYED: N23 and N24 are crossed to UV-sensitive mutants or to osmotic mutants. Mutants which carry ad-3A and the UV-sensitivity marker or ad-3A and the Osmotic marker are selected from the crosses. The mutagenic sensitivities of these mutants to chemical agents are tested according to the procedures previously described.

MAJOR FINDINGS AND PROPOSED COURSE: Preliminary results indicate that incorporation of the UVS-2 marker into N23 and N24 does not increase the sensitivity of these testers to mutagens. Whether incorporation of the Osmotic marker into these testers will increase the sensitivity has not yet been determined. Future efforts will include: (1) validation of these simple test systems; (2) extension of the in vitro activation system using N23 and N24 as indicator organisms; and (3) determination of whether addition of other UV-sensitivity markers increases the mutagenic sensitivity of the testers.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Development of this test system will provide a simple, economical, and quick system in eukaryotes for the detection and evaluation of the mutagenic activity of chemical agents in the human environment.

PUBLICATIONS

Ong, T.: Use of the spot, plate and suspension test systems for the detection of the mutagenicity of environmental agents and chemical carcinogens in Neurospora crassa. Mutation Res., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60005-05 LEM
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Mutagenicity of Chemotherapeutic Agents in <u>Neurospora crassa</u>		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: T. Ong Geneticist LEM NIEHS		
COOPERATING UNITS (if any) Miles Laboratory, Elkhart, Indiana		
LAB/BRANCH Laboratory of Environmental Mutagenesis		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline key words) Antischistosomal drugs and other chemotherapeutic agents are attracting wide concern because of their potential carcinogenic and mutagenic hazards to treated individuals in human populations. Our previous studies have shown that many antischistosomal agents are mutagenic in <u>Neurospora crassa</u> and that there is no correlation between mutagenicity and antischistosomal activity of antischistosomal agents. Studies have extended to other chemotherapeutic agents, and to determine whether antischistosomal agents and other chemotherapeutic agents induce particular types of genetic alterations in <u>N. crassa</u> . Genetic analysis of mutants induced by <u>niridazole</u> and <u>metronidazole</u> indicate that niridazole induces predominantly base-pair substitution mutations. None of the niridazole-induced mutants resulted from multilocus deletions. The spectrum of genetic alterations induced by metronidazole is similar to that induced by monofunctional alkylating agents.		

PROJECT DESCRIPTION

METHODS EMPLOYED: The ad-3 test system of N. crassa is used in this study. Conidia from a genetically marked two-component heterokaryon are treated with chemotherapeutic agents. The treated and untreated conidia are analyzed for the presence of ad-3 mutants by the direct method. The genotype and the complementation pattern of each mutant are determined by heterokaryon tests with various strains used as testers. Mutants are then tested by means of dikaryon and trikaryon tests to determine the frequency of chromosomal deletions.

MAJOR FINDINGS AND PROPOSED COURSE: Niridazole has been used for the treatment of schistosomiasis while metronidazole is widely used for the treatment of Trichomonas vaginalis. These two drugs are not mutagenic in resting conidia but are mutagenic in vegetative cultures of N. crassa. Genetic analysis of the mutants induced by these two compounds indicates that niridazole induces predominantly, if not exclusively, base-pair substitution mutations. None of the niridazole induced mutants resulted from multilocus deletions. The spectra of genetic alterations induced by metronidazole are similar to those induced by monofunctional alkylating agents. Further studies will be carried out with other compounds which are very closely related to human health.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Since antischistosomal agents and some other chemotherapeutic drugs are commonly used for the treatment of parasitic disease in man, it is important to determine the potential mutagenic hazards of these compounds to the treated individuals. Studies on the relationship between structure, mutagenicity and chemotherapeutic activity of drugs and structurally related compounds will be useful in the design of substances which can be used for the treatment of human disease.

PUBLICATIONS

Ong, T.: Mutagenic activities of nitrofurantoin in Neurospora crassa. Mutation Res. 56: 13-20, 1977.

Mohn, G., Ong, T., Callen, D.F. and Aaron, C.S.: Comparison of genetic activity of 5-nitroimidazole derivatives in Escherichia coli, Neurospora crassa and Saccharomyces cerevisiae. J. Toxicol. Envir. Health, in press.

Ong, T. and Slade, B.: Mutagenicity and mutagenic specificity of metronidazole and niridazole in Neurospora crassa. J. Toxicol. Envir. Health, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60026-05 LEM
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Comparative Sensitivities of Different Mouse Strains to Mutagenic Agents		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: W. Sheridan Mammalian Geneticist LEM NIEHS		
CODOPERATING UNITS (if any) E. Soares, Chemical Industry Institute of Toxicology		
LAB/BRANCH Laboratory of Environmental Mutagenesis		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.1	OTHER: 0.4
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline) Earlier studies, utilizing irradiation as the mutagenic source, have shown that the genome of the test organism is a major factor in the determination of its susceptibility to genetic damage. It is our purpose to investigate to what extent such genetically caused differences in sensitivity pertain to other mutagenic agents. We also want to know if the relative level of sensitivity of a defined genome to induction of mutations is constant for all agents or variable depending on the mutagen used. Preliminary screening of five <u>inbred strains</u> using <u>chemical mutagens</u> indicates the existence of strain differences among these strains of <u>mice</u> . Future efforts will include retests of these strains plus tests of additional strains. In addition, efforts will be devoted to understanding the underlying genetic causes of the differences.		

PROJECT DESCRIPTION

METHODS EMPLOYED: We want to accrue a battery of test strains, each showing a distinct level of sensitivity to mutation induction, covering a range from highly sensitive to highly resistant. These strains will be selected on the basis of induction of dominant lethal mutations and increases in the percent of morphologically aberrant sperm by known potent mutagens. They will then be challenged by a successive array of other known or suspected mutagenic agents to discover any changes in their relative levels of sensitivity.

MAJOR FINDINGS AND PROPOSED COURSE: Preliminary screening of five inbred strains has been made. There are indications of differences between some of the strains tested. These differences give promise of the success of the project; however, retests of these strains plus additional strains will be necessary before final choices are made of strains to be included in the test battery. Future efforts will be devoted to understanding the underlying genetic causes of differences in sensitivity to mutation induction and the consequences of combining the genetic materials of strains with divergent sensitivities.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Since the gene pools of human populations by and large are extremely heterogeneous, one would expect a broad spectrum of reactions to a mutagenic agent in the environment. Restriction of testing to single, often inbred, strains of another mammal such as the mouse will not give indications on a wide scale of what might be the effects of such an agent on the human population. By determining the effects of a compound on a battery of strains representing a variety of genotypes and differing sensitivities, we will be better able to approximate what might be expected in a heterogeneous mammalian population such as man.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60027-05 LEM
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Comparisons of Effects of Chemical Mutagens on Somatic and Germ Cells of Mice		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	W. Sheridan	Research Geneticist LEM NIEHS
Other:	R. Sorg	Geneticist LEM NIEHS
COOPERATING UNITS (if any) E. Soares, Chemical Industry Institute of Toxicology		
LAB/BRANCH Laboratory of Environmental Mutagenesis		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.7	PROFESSIONAL: 0.2	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline) This project is designed to explore the feasibility of using <u>somatic cells</u> to screen for chemically induced <u>chromosomal aberrations</u> . The use of somatic cells for screening purposes would allow for rapid screening of large numbers of animals provided such techniques are reliable in the detection of aberrations. Included in this project are comparisons with respect to the efficiency of detection of chromosome aberrations in <u>germ cells</u> (<u>testicular preparations</u> and <u>sperm morphology</u>), cultured <u>lymphocytes</u> and <u>bone marrow</u> preparations.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Lymphocytes cultured from offspring of treated males are prepared for cytological examination and karyotyping. Similarly, testicular sections and air dried preparations of testicular tissues as well as sperm from the vas and epididymis are examined for chromosome abnormalities and sperm head abnormalities respectively.

MAJOR FINDINGS AND PROPOSED COURSE: In the studies conducted thus far, chromosome preparations from cultured lymphocytes or bone marrow seem to be less reliable for use in the detection of aberrations than chromosomes prepared from testicular tissues. Lymphocyte preparations are most useful in those cases where the chromosomal aberration (translocation) results in the production of (1) small fragments, (2) metacentric chromosomes or (3) chromosomes with disproportionately large or small lengths relative to the control karyotypes. In functionally sterile males suspected to be translocation carriers, we found upon examination of sperm that (1) actual counts of sperm were severely reduced and/or (2) as many as 90% of the sperm present were abnormally shaped. The mutagenic effects in mice of chemical agents will be tested. The mode of action and the long-term effects of induced, heritable chromosomal abnormalities will be studied.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Although chromosome abnormalities are known to exist and be transmitted to following generations, very little is known of the long-term effects of such aberrations. Studies of the effects of heritable chromosome aberrations in mice will foster a better understanding of the mode of action and significance to the well being of the animal of such abnormalities.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60038-03 LEM
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Aflatoxin Mutagenesis in <u>Neurospora crassa</u>		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: T. Ong Geneticist LEM NIEHS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Environmental Mutagenesis		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline !) Aflatoxin B ₁ is one of the most potent chemical <u>carcinogens</u> found in the human environment. This and other aflatoxins are common contaminants in agricultural products. Our previous studies have shown that aflatoxin B ₁ and G ₁ can be converted by hamster, rat or mouse liver homogenates to metabolites which are <u>mutagenic in Neurospora</u> , and that aflatoxin B ₁ is much more mutagenic than aflatoxin G ₁ in an <u>in vitro activation</u> system using liver or kidney homogenates from rats or hamsters. Further studies on the relationship between carcinogenesis and mutagenesis and the mechanism of mutagenesis show that aflatoxin B ₁ <u>per se</u> appears to be a weak mutagen in the resting conidia of the ultraviolet-repair-deficient strain UVS-2 of <u>Neurospora</u> . It seems that aflatoxin B ₁ causes certain genetic damage which can be repaired by the wild type but not by the UV-sensitive strain.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Conidia from a genetically marked two-component heterokaryon of a wild type strain (H12) and of an excision-repair-deficient strain (H59) are harvested and treated with aflatoxin B₁ at 37°C for 2 hrs. At the end of the treatment, conidia are washed. Treated and untreated conidia are assayed for the presence of ad-3 mutants by the direct method.

MAJOR FINDINGS AND PROPOSED COURSE: Aflatoxin B₁ is not mutagenic in the resting conidia of wild type Neurospora. This compound per se appears to be a weak mutagen in the UV-repair-deficient strain UVS-2 of N. crassa. It seems, therefore, that aflatoxin B₁ causes certain genetic damage which can be repaired by the wild type but not by the UV-sensitive strain which is defective in excision repair. In the future, attempts will be made to identify the active metabolites of aflatoxins by either genetic or biochemical studies.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Aflatoxins are common contaminants in agricultural products. These compounds, therefore, are very closely related to human health. Studies described in this project will provide information in regard to the potential mutagenic hazard of these mycotoxins, the mechanism of aflatoxin mutagenesis, and the relationship between carcinogenesis and mutagenesis of these compounds.

PUBLICATIONS

Callen, D., Mohn, G. and Ong, T.: Comparison of genetic activity of aflatoxin B₁ and G₁ in Escherichia coli and Saccharomyces cerevisiae. Mutation Res. 45: 7-11, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60042-03 LEM										
PERIOD COVERED October 1, 1977 to September 30, 1978												
TITLE OF PROJECT (80 characters or less) The Study of Recessive Lethal Mutations Induced in Mice by Chemical Mutagens												
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT												
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">W. Sheridan</td> <td style="width: 20%;">Research Geneticist</td> <td style="width: 10%;">LEM</td> <td style="width: 15%;">NIEHS</td> </tr> <tr> <td>Other:</td> <td>M. Segall</td> <td>Geneticist</td> <td>LEM</td> <td>NIEHS</td> </tr> </table>			PI:	W. Sheridan	Research Geneticist	LEM	NIEHS	Other:	M. Segall	Geneticist	LEM	NIEHS
PI:	W. Sheridan	Research Geneticist	LEM	NIEHS								
Other:	M. Segall	Geneticist	LEM	NIEHS								
COOPERATING UNITS (if any) None												
LAB/BRANCH Laboratory of Environmental Mutagenesis												
SECTION												
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709												
TOTAL MANYEARS: 1.3	PROFESSIONAL: 0.5	OTHER: 0.8										
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS												
SUMMARY OF WORK (200 words or less - underline)												
<u>Studies are being conducted of the induction of recessive lethal mutations in mice by chemical mutagens.</u> Backcross progeny between F_1 offspring of <u>chemical mutagen</u> treated parents and their F_2 daughters are used to detect the <u>presence of recessive lethals</u>. An increase in fetal mortality is used as indicator of the lethal. The induction of recessive lethal mutations by irradiation has previously been demonstrated in the mouse. No adequate study of this class of mutation has been conducted in mice following treatment with chemical mutagens. It is our purpose to investigate whether chemicals will induce recessive lethals in mice and what might be the mode of action and long-term effects of such mutations on mammals.												

PROJECT DESCRIPTION

METHODS EMPLOYED: Inbred male mice of the CBA strain were treated with a known chemical mutagen (TEM) and mated to untreated females of the same strain. F_1 male progeny were collected and individually mated to females of the strain. A series of backcrosses between F_2 females and their sires were conducted to determine whether there was an increase in fetal mortality indicative of the presence of a recessive lethal.

MAJOR FINDINGS AND PROPOSED COURSE: Preliminary analyses of the data indicates that a high frequency of the F_1 males studied are bearers of recessive lethals. Comparison of pedigrees gives presumptive evidence that the greater proportion of these lethals were induced by the mutagenic treatment. Future studies will include investigations of the effects of recessive lethals on early developmental stages in homozygotes, comparisons of heterozygotes with normal homozygotes and attempts at biochemical identification of the loci in question.

SIGNIFICANCE TO BIOCHEMICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It is estimated that on the average the human genome contains 5 deleterious recessive genes. Although there are known examples of genes which have an extreme effect in the homozygote also showing an effect in the heterozygote, for the most part little is known about the mode of action or long-term effects on the bearer of such genes. Studies are necessary to determine frequencies of induction of this class of genes by mutagenic agents and their consequences to the individual.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60049-02 LEM															
PERIOD COVERED October 1, 1977 to September 30, 1978																	
TITLE OF PROJECT (80 characters or less) The Role of Enzyme Induction in the Metabolism of Mutagens																	
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">E. Zeiger</td> <td style="width: 35%;">Research Microbiologist</td> <td style="width: 10%;">LEM</td> <td style="width: 5%;">NIEHS</td> </tr> <tr> <td>Other:</td> <td>R. Chhabra</td> <td>Senior Staff Fellow</td> <td>EBCB</td> <td>NIEHS</td> </tr> <tr> <td></td> <td>B. Margolin</td> <td>Statistician</td> <td>BB</td> <td>NIEHS</td> </tr> </table>			PI:	E. Zeiger	Research Microbiologist	LEM	NIEHS	Other:	R. Chhabra	Senior Staff Fellow	EBCB	NIEHS		B. Margolin	Statistician	BB	NIEHS
PI:	E. Zeiger	Research Microbiologist	LEM	NIEHS													
Other:	R. Chhabra	Senior Staff Fellow	EBCB	NIEHS													
	B. Margolin	Statistician	BB	NIEHS													
COOPERATING UNITS (if any) Environmental Biology and Chemistry Branch, NIEHS Biometry Branch, NIEHS																	
LAB/BRANCH Laboratory of Environmental Mutagenesis																	
SECTION																	
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																	
TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.4	OTHER: 0															
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																	
SUMMARY OF WORK (200 words or less - underline keywords) The effects of Arochlor-1254 induction on the levels of rat hepatic enzymes, and on the ability of induced liver preparations to metabolize <u>benzo(a)pyrene</u> (BaP) and 2-aminoanthracene (2-AA) to mutagens was studied. Arochlor treatment induced <u>cytochrome P488/450</u> , <u>arylhydrocarbon hydroxylase</u> (AHH), <u>aniline hydroxylase</u> , <u>aminopyrine N-demethylase</u> and <u>DMN-N-demethylase</u> in both Sprague-Dawley and Fisher-344 rats. Arochlor treatment enhanced the mutagenicity of BaP for <u>Salmonella typhimurium</u> TA-98 but depressed the mutagenicity of 2-AA. Freezing and storage of the liver preparations affected neither enzymic activity nor metabolic activation of BaP or 2-AA.																	

PROJECT DESCRIPTION

METHODS EMPLOYED: Male Sprague-Dawley and Fisher-344 rats were treated IP with 500 mg/kg Arochlor 1254 in corn oil, and sacrificed at 1, 3, 5 and 7 days after treatment; control rats were treated with corn oil only. 9000 Xg supernatant fractions (S-9) prepared from homogenized livers were used for enzyme assays and mutagen activation. Mutagenesis studies were done by the Ames procedure using 3 concentrations of S-9 per plate.

MAJOR FINDINGS AND PROPOSED COURSE: Arochlor induction significantly increased levels of all enzymes studied, but protein/gm liver was unchanged. There was no day-related increase in AHH or cytochrome activities, while the other enzyme activities showed peak responses at about 5 days past induction. BaP mutagenesis was enhanced and 2-AA mutagenesis was depressed by S-9 from induced animals. There was a positive correlation with BaP mutagenicity as a function of AHH or cytochrome concentration; 2-AA produced a negative correlation. Additional studies will be performed to determine if the enzyme induction and mutagenic responses are also present in extrahepatic tissues and other animal species, and whether the depression of mutagenicity seen with 2-AA is also seen with other aromatic primary amines.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Arochlor-induced rat liver S-9 is widely used for metabolic activation of chemicals tested for mutagenicity. This study is designed to define the enzymic properties of Arochlor-induced S-9 and also to evaluate the suitability of induced versus uninduced S-9 for mutagenic studies.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES-60050-02 LEM
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Evaluation of Metabolic Activation Techniques for Mutagens for Yeast		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Other:	D. Frezza E. Zeiger N. Gupta B. Smith	Visiting Fellow Research Microbiologist Pathologist Staff Fellow LEM LEM EBCB LP NIEHS NEIHS NIEHS NIEHS
COOPERATING UNITS (if any) Environmental Biology and Chemistry Branch, NIEHS Laboratory of Pharmacology, NIEHS		
LAB/BRANCH Laboratory of Environmental Mutagenesis		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (a1) MINORS ☐ (a2) INTERVIEWS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER		
SUMMARY OF WORK (200 words or less - underline) The intrasanguineous host-mediated assay in mice and rats and perfused rat liver and lung were examined as systems for <u>metabolizing mutagens</u> for <u>Saccharomyces cerevisiae</u> D4. There was no observable histological damage in the liver, lungs, kidneys, and testes up to 3 days after I.V. injection of yeast into mice. The yeast cells were trapped in the capillaries of the various organs, with a few phagocytized cells. The metabolism of <u>dimethylnitrosamine</u> (DMN) to a mutagen was compared in the host-mediated assay, perfused liver, and liver homogenate systems. Mice were more effective than rats in converting <u>DMN</u> to a mutagen and the host mediated and perfusion techniques were more effective than liver homogenates <u>in vitro</u> .		

PROJECT DESCRIPTION

METHODS EMPLOYED: Mice were injected in the tail vein with approximately 1×10^8 cells and were sacrificed at varying time intervals. Tissues were prepared for histological analysis and other mice were used to measure the efficiency of yeast recovery from the different organs. Rat liver and lung preparations were perfused for 2 hours.

MAJOR FINDINGS AND PROPOSED COURSE: Up to 3 days after injection with yeast, the mice exhibited no histological damage in the liver, lungs, kidneys or testes. Upon histological examination the yeast were seen to be trapped in the capillaries of the various organs with a small amount of phagocytation by macrophages. The highest recovery of the yeast was from the liver; adequate numbers of cells could be recovered from the lungs and kidneys. DMN was mutagenic in the liver, lungs and kidneys in the host-mediated assay. It was also mutagenic in perfused liver but no mutagenicity was seen in perfused lung. Organ homogenates were all capable of converting DMN to a mutagen. Although it is difficult to compare doses between the various test systems, in vitro organ homogenates appeared to produce a lower mutagenic effect than the host-mediated assay.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The intrasanguineous host mediated assay may be a valuable technique for studying the in vivo distribution of genetically active substances in mice and rats. This study provides information on the organ and tissue distribution of the yeast, the time course of this distribution, and organ-specific metabolism. This work also demonstrates that a perfused organ system can be used in combination with a microbial system for mutagenesis studies.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60052-02 LEM																																			
PERIOD COVERED October 1, 1977 to September 30, 1978																																					
TITLE OF PROJECT (80 characters or less) Environmental Mutagenesis Test Development Program																																					
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																																					
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">L.R. Valcovic</td> <td style="width: 30%;">Geneticist</td> <td style="width: 10%;">LEM</td> <td style="width: 10%;">NIEHS</td> </tr> <tr> <td>Other:</td> <td>E. Zeiger</td> <td>Research Microbiologist</td> <td>LEM</td> <td>NIEHS</td> </tr> <tr> <td></td> <td>T. Ong</td> <td>Geneticist</td> <td>LEM</td> <td>NIEHS</td> </tr> <tr> <td></td> <td>G. Hoffmann</td> <td>Geneticist</td> <td>LEM</td> <td>NIEHS</td> </tr> <tr> <td></td> <td>D. Walters</td> <td>Chemist</td> <td>EBCB</td> <td>NIEHS</td> </tr> <tr> <td></td> <td>B. Margolin</td> <td>Statistician</td> <td>BB</td> <td>NIEHS</td> </tr> <tr> <td></td> <td>D. Smith</td> <td>Computer-programmer</td> <td>BB</td> <td>NIEHS</td> </tr> </table>			PI:	L.R. Valcovic	Geneticist	LEM	NIEHS	Other:	E. Zeiger	Research Microbiologist	LEM	NIEHS		T. Ong	Geneticist	LEM	NIEHS		G. Hoffmann	Geneticist	LEM	NIEHS		D. Walters	Chemist	EBCB	NIEHS		B. Margolin	Statistician	BB	NIEHS		D. Smith	Computer-programmer	BB	NIEHS
PI:	L.R. Valcovic	Geneticist	LEM	NIEHS																																	
Other:	E. Zeiger	Research Microbiologist	LEM	NIEHS																																	
	T. Ong	Geneticist	LEM	NIEHS																																	
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	D. Smith	Computer-programmer	BB	NIEHS																																	
COOPERATING UNITS (if any) Environmental Biology & Chemistry Branch, NIEHS Biometry Branch, NIEHS																																					
LAB/BRANCH Laboratory of Environmental Mutagenesis																																					
SECTION																																					
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																																					
TOTAL MANYEARS: 3.7	PROFESSIONAL: 2.7	OTHER: 1.0																																			
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (a1) MINORS ☐ (a2) INTERVIEWS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER																																					
SUMMARY OF WORK (200 words or less - underline)																																					
The primary objective of this effort is to develop a program which will have the ultimate capability of <u>testing the mutagenicity</u> of 1000 chemicals per year using <u>microbial systems</u>, and an as-yet-undetermined smaller number of chemicals in higher <u>in vitro</u> and <u>in vivo</u> test systems. The major component of this development process will be <u>test system validation</u>, which includes; (1) <u>reproducibility</u>, (2) <u>comparison with other mutagenicity tests</u>, (3) <u>comparison of the carcinogenesis tests</u>, and (4) <u>structure/activity relationships</u>. Since the volume of data generated in this program will be <u>large</u>, an important ancillary aspect will be the development of <u>computer holding</u> and <u>processing procedures</u>.																																					

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: Microbial Testing - One contract is already in effect, and provides for the testing of about 100 chemicals per year. It is anticipated that two or three additional awards will be made in FY 78, each consisting of 75 to 100 compounds per year. The speed with which we approach our maximum will depend strongly upon how successful are these initial efforts in establishing the effectiveness of the tests in the field, and upon the types of difficulties which arise (impossible to anticipate, but sure to come in an unprecedented undertaking of this magnitude).

Chemicals - Selection of test compounds will be made by an NIEHS committee from candidate compounds used by industry or the consumer. Close contact will be maintained with other government agencies and the staffs of their mutagenesis testing programs, and provision may be made for individual agencies to specify up to certain prearranged numbers of compounds for inclusion in the NIEHS testing program. A contract will be let to a single laboratory to locate and purchase chemicals selected for testing. This contractor will also be responsible for storing and dispensing the chemicals to the testing laboratories, and will develop a computerized inventory of the compounds, including such information as CAS Number, Wiswesser linear notation, lot number, and analytical information, as well as all available information on toxicity.

Data Handling - In collaboration with biometry personnel, work is progressing on the development of a data management system and statistical evaluations of microbial test data. In addition, a contract has been awarded to develop a computer-programmed protocol for conducting and interpreting microbial tests.

The information generated will serve little purpose unless it can be made available to interested individuals or agencies. To this end, mechanisms will be developed for data distribution. A number of mechanisms either already exist or are in development. (1) The Environmental Mutagen Information Center (EMIC) is developing a file of chemicals tested for mutagenicity. The chemicals and the systems in which they were tested can be entered into EMIC and thereby be made available to EMIC users. (2) The DHEW Committee to Coordinate Toxicology and Related Programs can be used as a vehicle for rapid, informal distribution of test results. (3) Test results could be published in regular or special issues of Environmental Health Perspectives and other scientific journals. (4) Results can also be published by the National Technical Information Service.

The test program will be designed as a blind study for the testing and data evaluation segments of the program. Therefore, the code on any individual chemical will not be broken until after the testing is completed, the data evaluated, and a decision made on the mutagenicity of the chemical. This will avoid dissemination of invalid or incomplete data.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

There are literally thousands of chemicals in use and the number is ever increasing. The majority of these substances has not been evaluated for the potential to induced heritable genetic alterations; and, hence, the possible risk to future human generations is unknown. This program will develop an efficient coordinated system for mutagenesis testing and should serve as a model system. The data obtained from this program will be useful for decision making on the safety of various classes of chemicals to which man is exposed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60053-02 LEM
PERIOD COVERED <u>October 1, 1977 to September 30, 1978</u>		
TITLE OF PROJECT (80 characters or less) Mutagenicity of Dimethylnitrosamine in <u>Neurospora crassa</u>.		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Other:	W. Whong T. Ong	Visiting Fellow Geneticist
		LEM NIEHS LEM NIEHS
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Environmental Mutagenesis		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0
CHECK APPROPRIATE BOX(ES)		
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER		
☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline 1 <u>Dimethylnitrosamine (DMN)</u> , a potent procarcinogen, is not mutagenic <u>per se</u> in <u>Neurospora crassa</u> conidia because the conidia seem to be deficient in the activation process for promutagens. With metabolic activation, however, this agent is an effective mutagen in this organism. With the ad-3 system of <u>N. crassa</u> , systematic studies are carried out on four metabolic activation systems (growth-mediated, mycelium extract-mediated, organ homogenate-mediated, and host-mediated), for converting DMN to a mutagen. In addition, DMN activation by both sexes, and by organs (liver, lung and kidney are also compared, both <u>in vitro</u> and <u>in vivo</u> .		

PROJECT DESCRIPTION

METHODS EMPLOYED: The ad-3 forward-mutation analysis (direct method) in a UV-sensitive heterokaryon (H-59) and the reverse-mutation analysis (standard method) in an ad-3A presumptive base-pair substitution mutant (N-23) of N. crassa were employed. For growth activation, DMN was added directly to culture medium. Conidia of vegetative cultures growing from this medium were used for determining the mutation frequency. For mycelium extract-mediated and organ homogenate-mediated activations, conidia of N. crassa were cotreated with S-9 fraction of mycelia or animal organs and DMN. In host-mediated activation, conidia and DMN were administered by intravenous and intramuscular injections, respectively.

MAJOR FINDINGS AND PROPOSED COURSE: DMN was effectively mutagenic in N. crassa conidia in all activation systems tested. However, DMN activation was much higher with in vivo (growth- and host-mediated) than with in vitro (mycelium extract- and organ homogenate-mediated) activations. In regard to the in vitro study on both sexes of mice and rats, the organ-activating potency for DMN was in the order: lung > kidney > liver. However, there was a reverse order among these three organs of the male mouse and both sexes of the rat for the activation of DMN in host-mediated activation. A sex difference in the activation of DMN was seen only in the mouse kidney (the male was more active) with the host-mediated activation. The ad-3 mutants of N. crassa recovered from the organs with host-mediated activation will be genetically characterized by complementation analyses.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Results from these studies provide further understanding of the metabolic activation of promutagens. The comparative studies on the activation of DMN by various organs of different animal species also provides information on the relationship between the mutagenicity and carcinogenicity of this environmental procarcinogen (DMN), and the effectiveness of in vitro as compared to in vivo activation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60057-02 LEM
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Molecular Analyses of Mitochondrial DNAs of Different Species of <u>Drosophila</u>		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: C. Langley D. Shah	Research Geneticist Visiting Fellow	LEM NIEHS LEM NIEHS
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Environmental Mutagenesis		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 1.1	PROFESSIONAL: 1.1	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline key words) We have done preliminary characterization of mitochondrial DNAs isolated from three species of <u>Drosophila</u> . Electron microscope heteroduplex and <u>restriction endonuclease analyses</u> have revealed that these mtDNAs have undergone a significant <u>evolutionary divergence</u> in their nucleotide sequences. We are constructing specific cleavage maps of these mtDNAs using various restriction endonucleases. Restriction endonucleases recognize specific base sequences within a DNA molecule and have proven useful in mapping genes and detecting single base-pair substitutions. Utilizing this information, our objectives are to understand (1) organization of genes as related to function, (2) how the gene arrangements change, (3) the rates of evolution of small DNAs, and (4) heterogeneity of intra-specific mtDNA.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Electron microscope heteroduplex analysis and restriction endonuclease cleavage analysis are currently being used to estimate the nucleotide sequence divergence in the circular mitochondrial DNAs of different species of Drosophila.

MAJOR FINDINGS AND PROPOSED COURSE: Size heterogeneity has been detected in the mitochondrial DNAs of distantly related species of Drosophila. Electron microscope partial denaturation mapping has revealed the presence of adenine-thymine rich regions in the mitochondrial DNAs of Drosophila. The length of the AT-rich region varies in different species. Heteroduplex analysis has shown the AT-rich region to be the evolutionarily most pliable region in the mitochondrial DNA of Drosophila. Specific cleavage sites of four different restriction endonucleases have been mapped on mitochondrial DNAs of three species of Drosophila. Some important information has been obtained from the restriction endonuclease cleavage maps concerning the evolution of these mitochondrial DNAs. The technique of restriction endonuclease cleavage mapping will be further extended to estimate the nucleotide sequence divergence of mitochondrial DNA within species.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Genetic variation in mitochondrial DNA of higher eukaryotic organisms must be understood in order to judge the consequences of mutational damage. Very little is known about the extent and nature of genetic variation in mitochondrial chromosomes of animals. This project will provide some basic information and hopefully provide some evidence for the role of newly arising mutants.

PUBLICATIONS

Shah, D.M. and Langley, C.H.: Complex mitochondrial DNA in Drosophila. Nucleic Acids Research. 4: 2949-2960, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60058-02 LEM																				
PERIOD COVERED October 1, 1977 to September 30, 1978																						
TITLE OF PROJECT (80 characters or less) Amount and Effects of Null Allozymic Variation in Natural Populations																						
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">C. Langley</td> <td style="width: 30%;">Research Geneticist</td> <td style="width: 10%;">LEM</td> <td style="width: 10%;">NIEHS</td> </tr> <tr> <td>Other:</td> <td>R. Voelker</td> <td>Senior Staff Fellow</td> <td>LEM</td> <td>NIEHS</td> </tr> <tr> <td></td> <td>A. Leigh Brown</td> <td>Visiting Fellow</td> <td>LEM</td> <td>NIEHS</td> </tr> <tr> <td></td> <td>S. Ohnishi</td> <td>Visiting Fellow</td> <td>LEM</td> <td>NIEHS</td> </tr> </table>			PI:	C. Langley	Research Geneticist	LEM	NIEHS	Other:	R. Voelker	Senior Staff Fellow	LEM	NIEHS		A. Leigh Brown	Visiting Fellow	LEM	NIEHS		S. Ohnishi	Visiting Fellow	LEM	NIEHS
PI:	C. Langley	Research Geneticist	LEM	NIEHS																		
Other:	R. Voelker	Senior Staff Fellow	LEM	NIEHS																		
	A. Leigh Brown	Visiting Fellow	LEM	NIEHS																		
	S. Ohnishi	Visiting Fellow	LEM	NIEHS																		
COOPERATING UNITS (if any) None																						
LAB/BRANCH Laboratory of Environmental Mutagenesis																						
SECTION																						
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																						
TOTAL MANYEARS: 4.5	PROFESSIONAL: 3.7	OTHER: 0.8																				
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																						
SUMMARY OF WORK (200 words or less - underline key words) Virtually nothing is known about the frequency of "null" allozyme alleles (alleles which code for the production of no enzyme activity) in natural populations or their importance to the well being of the individuals who carry such alleles. This study seeks to answer these questions for several natural populations of <u>Drosophila melanogaster</u> . Partial analysis of a sample from the Raleigh, North Carolina population of <u>Drosophila melanogaster</u> suggests that the frequency of "null" allozyme alleles is less than one percent per locus and varies considerably among loci.																						

PROJECT DESCRIPTION

METHODS EMPLOYED: Extraction of chromosomes from widely separated natural populations utilizes dominantly marked balancer chromosomes. Electrophoretic assays utilize gel electrophoresis preparation and staining techniques. Cytological analyses involve examination and photography of salivary gland chromosome preparations. Immunologic assays involve preparations of antibodies to purified wild-type enzymes and detection of cross-reacting materials.

MAJOR FINDINGS AND PROPOSED COURSE: A sample of between 800 and 1000 gametes from the North Carolina population of *Drosophila melanogaster* is being screened for twenty-six loci having the following chromosomal distribution: X chromosome (5 loci), second chromosome (9 loci) and third chromosome (12 loci). No nulls or low-activity mutants have been found on the X chromosome. Putative nulls have been found at the following loci on the second chromosome: Pgi (1); Got (2); αGpdh (8); Hex C (1); and Adh (6). These are in the process of being verified and analyzed as described above. Partial results of screens of the third chromosome loci have yielded five nulls, three at Aldox and two at Me. The ALDOX nulls are viable over a cytogenetic deficiency for Aldox and are being analyzed for CRM production. The ME nulls are viable over a cytogenetic deficiency for Me and are CRM negative. Cytogenetic analyses of these nulls will be completed soon.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The information obtained from this study is vital to an understanding of the genetic variation which exists in natural populations, both as regards to amount and type (i.e., base substitutions, deletions). Once the amount and types of "natural variation are known, it will be possible to assess the effects attributable to new environmental mutagens. Moreover, the biological effect of homozygosity for "null" alleles, based on limited evidence, presents a conflicting picture; in humans such effects are thought to be severely detrimental, while in *Drosophila* the effects appear to be less drastic. By detecting a large number of "nulls" at many loci, it should be possible to draw more accurate conclusions for *Drosophila* and ultimately for mammals, including man.

PUBLICATIONS

Voelker, R. A., and Langley, C. H.: Cytological localization of Roi (rough eye). *Drosophila* Information Service. 52, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60059-02 LEM										
PERIOD COVERED October 1, 1977 to September 30, 1978												
TITLE OF PROJECT (80 characters or less) Effects of Chromosomal Aberrations on Embryonic Development												
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">W. Sheridan</td> <td style="width: 20%;">Research Geneticist</td> <td style="width: 10%;">LEM</td> <td style="width: 20%;">NIEHS</td> </tr> <tr> <td>Other:</td> <td>H. Michelmann</td> <td>Visiting Fellow</td> <td>LEM</td> <td>NIEHS</td> </tr> </table>			PI:	W. Sheridan	Research Geneticist	LEM	NIEHS	Other:	H. Michelmann	Visiting Fellow	LEM	NIEHS
PI:	W. Sheridan	Research Geneticist	LEM	NIEHS								
Other:	H. Michelmann	Visiting Fellow	LEM	NIEHS								
COOPERATING UNITS (if any) None												
LAB/BRANCH Laboratory of Environmental Mutagenesis												
SECTION												
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709												
TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.6	OTHER: 0										
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS												
SUMMARY OF WORK (200 words or less - underline key words) This project is designed to study the effects of <u>chromosomal aberrations</u> on <u>fetal mortality</u> and <u>development</u>. Comparisons will be made between <u>in vivo</u> effects and effects on <u>mouse embryos</u> grown in <u>culture</u>. Chemical mutagens are known to be capable of inducing chromosomal aberrations such as translocations which may be transmitted to the next generation. Increased rates of fetal death are usually observed among offspring to translocation heterozygotes. It is our purpose to investigate the rates of transmission of such aberrations and the processes of fetal mortality by direct observation of the development of embryos.												

PROJECT DESCRIPTION

METHODS EMPLOYED: Known translocation-bearing mice are mated and the F_1 sons studied to determine the frequency of translocation heterozygotes. In other matings the females are euthanized shortly after fertilization, and two cell embryos are retrieved from the oviducts and established in culture. Stage of development arrest is determined.

MAJOR FINDINGS AND PROPOSED COURSE: F_2 males are currently being analyzed to determine whether they are translocation heterozygotes. Cytogenetic analysis is being conducted in attempts to correlate the nature of the aberration and the effect on embryonic development.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Chromosome aberrations in man are known to cause disturbances in development of embryos leading to death of the embryo or to abnormal offspring. An understanding of the fate and effects of chromosome aberrations which may be caused by environmental agents is necessary for evaluation of the possible risks such induced aberrations may imply for man.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60062-02 LEM
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Molecular Characterization of Isozymes and Mutant Enzymes in Mammals		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Other:	S. Li S.-M. Chang S. Huang C.-Y. Lee	Research Geneticist Visiting Fellow Visiting Fellow Senior Staff Fellow
		LEM NIEHS LEM NIEHS LEM NIEHS LEM NIEHS
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Environmental Mutagenesis		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 2.0	PROFESSIONAL: 2.0	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline key words) The <u>amino acid composition</u> and <u>tryptic peptide maps</u> of three isozymes, LDH-M, LDH-H, and LDH-X, purified from mouse muscle, heart, and testis, respectively, have been compared. The testicular isozyme LDH-X has also been purified from both rat and hamster, and its <u>amino acid composition</u> determined in each case. Isozyme LDH-M has also been purified from horse muscle, and its <u>enzymatic properties</u> , <u>amino acid composition</u> and <u>tryptic peptide map</u> determined.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Lactate dehydrogenases are purified by AMP-affinity chromatography. Tryptic peptide maps are analyzed on cellulose thin-layer plates by electrophoresis and chromatography.

MAJOR FINDINGS AND PROPOSED COURSE: Three homotetrameric lactate dehydrogenase isozymes, LDH-M (A_4), LDH-H (B_4), and LDH-X (C_4), have been purified from mouse muscle, heart and testis, respectively. Based upon the amino acid composition and tryptic peptide maps of three isozymes, subunits A (muscle) and B (heart) appear to be more closely related to each other than to subunit C (testis). Two other testicular isozymes, LDH-X, have been purified from rat and hamster, and their amino acid composition and tryptic peptide maps are being compared. The primary structure variation of these testicular isozymes will be correlated with their antigenic properties. Isozyme LDH-M has also been purified from horse muscle. The enzymatic properties, amino acid composition and tryptic peptide map have been determined and compared with those of other mammalian LDH isozymes.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Primary structure characterization of various lactate dehydrogenase isozymes indicates the evolutionary relationship of different LDH genes. Molecular analysis of mutant proteins will elucidate the nature of genetic mutations in mammals.

PUBLICATIONS

Chang, S. M., C.-Y. Lee, R. Chen, and S. Li: Compositional relatedness of lactate dehydrogenase isozymes from mouse muscle, heart, and testis. Federation Proceeding 37, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-ES 60063-02 LEM																				
PERIOD COVERED <u>October 1, 1977 to September 30, 1978</u>																						
TITLE OF PROJECT (80 characters or less) Amino Acid Sequence of Mouse Sperm Lactate Dehydrogenase																						
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">J. Marciniszyn</td> <td style="width: 30%;">Senior Staff Fellow</td> <td style="width: 10%;">LEM</td> <td style="width: 10%;">NIEHS</td> </tr> <tr> <td>Other:</td> <td>Y.-C. Pan</td> <td>Visiting Fellow</td> <td>LEM</td> <td>NIEHS</td> </tr> <tr> <td></td> <td>S. Huang</td> <td>Visiting Fellow</td> <td>LEM</td> <td>NIEHS</td> </tr> <tr> <td></td> <td>S. Li</td> <td>Research Geneticist</td> <td>LEM</td> <td>NIEHS</td> </tr> </table>			PI:	J. Marciniszyn	Senior Staff Fellow	LEM	NIEHS	Other:	Y.-C. Pan	Visiting Fellow	LEM	NIEHS		S. Huang	Visiting Fellow	LEM	NIEHS		S. Li	Research Geneticist	LEM	NIEHS
PI:	J. Marciniszyn	Senior Staff Fellow	LEM	NIEHS																		
Other:	Y.-C. Pan	Visiting Fellow	LEM	NIEHS																		
	S. Huang	Visiting Fellow	LEM	NIEHS																		
	S. Li	Research Geneticist	LEM	NIEHS																		
COOPERATING UNITS (if any) None																						
LAB/BRANCH Laboratory of Environmental Mutagenesis																						
SECTION																						
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, NC 27709																						
TOTAL MANYEARS: 3.0	PROFESSIONAL: 2.8	OTHER: 0.2																				
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																						
SUMMARY OF WORK (200 words or less - underline keywords) The sperm-specific lactate dehydrogenase from mouse has been cleaved into small peptides by CNBr and trypsin. These peptides are being purified by gel filtration and ion-exchange chromatography. Amino acid sequences of some purified peptides are being determined.																						

PROJECT DESCRIPTION

METHODS EMPLOYED: Sperm-specific lactate dehydrogenase from mouse testis has been purified by AMP-affinity chromatography, and fragmented into peptides by chemical (CNBr) and enzymatic (trypsin) cleavage. These peptides are being separated by gel filtration and ion-exchange chromatography. Amino acid sequences of the pure peptides are being determined by automatic Edman degradation on a liquid-phase protein sequencer.

MAJOR FINDINGS AND PROPOSED COURSE: The CNBr fragments and tryptic peptides of mouse LDH-X are being separated and the amino acid sequences of some pure peptides are being determined. The complete primary structure will be established by overlapping various peptide sequences. The submolecular fragments of this LDH-X will also be used to study the antigenic properties of this enzyme.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

The complete amino acid sequence of this sperm-specific lactate dehydrogenase from mouse will be correlated with the results obtained from X-ray studies regarding the structure-function relationship of this enzyme. Elucidation of the primary structure of this enzyme is essential for studies of the antigenic properties of lactate dehydrogenases. This sperm-specific enzyme will be used for monitoring of mutations caused by environmental mutagens in human populations.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-ES 60064-02 LEM										
PERIOD COVERED October 1, 1977 to September 30, 1977												
TITLE OF PROJECT (80 characters or less) Primary Structure Characterization of Mouse Muscular Lactate Dehydrogenase												
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%;"> <tr> <td style="width: 33%;">PI:</td> <td style="width: 33%;">Y. Nakashima</td> <td style="width: 33%;">Visiting Scientist</td> <td style="width: 10%;">LEM</td> <td style="width: 10%;">NIEHS</td> </tr> <tr> <td>Other:</td> <td>S. Li</td> <td>Research Geneticist</td> <td>LEM</td> <td>NIEHS</td> </tr> </table>			PI:	Y. Nakashima	Visiting Scientist	LEM	NIEHS	Other:	S. Li	Research Geneticist	LEM	NIEHS
PI:	Y. Nakashima	Visiting Scientist	LEM	NIEHS								
Other:	S. Li	Research Geneticist	LEM	NIEHS								
COOPERATING UNITS (if any) None												
LAB/BRANCH Laboratory of Environmental Mutagenesis												
SECTION												
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709												
TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.3	OTHER: 0.2										
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS												
SUMMARY OF WORK (200 words or less - underline keywords) Lactate <u>dehydrogenase</u> from <u>mouse muscle</u> has been fragmented into small peptides by chemical and enzymatic procedures. These peptides are being purified by gel filtration and ion-exchange chromatography. <u>Amino acid sequences</u> of some pure peptides are being determined.												

PROJECT DESCRIPTION

METHODS EMPLOYED: Lactate dehydrogenase from mouse muscle has been purified by affinity chromatography. The purified enzyme has been cleaved into small peptides by chemical (CNBr) and enzymatic (trypsin and Staphylococcal protease) methods. These peptides are being isolated by gel filtration and ion-exchange chromatography. Amino acid sequences of pure peptides are being determined with an automatic protein sequencer

MAJOR FINDINGS AND PROPOSED COURSE: The CNBr fragments of this enzyme (LDH-M) are separated by gel filtration. Some pure peptides have been sequenced. The small peptides derived from protease digestion are being purified. The amino acid composition of the pure peptides is being determined. The total amino acid sequence of LDH-M will be established from several overlapping peptide sequences.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The amino acid sequence differences among various isozymes of lactate dehydrogenases will be correlated with their antigenic properties. This information is essential for a genetic monitoring program using mono-specific antibodies of the sperm-specific enzyme as a mutation marker.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60067-01 LEM										
PERIOD COVERED October 1, 1977 to September 30, 1978												
TITLE OF PROJECT (80 characters or less) Development of a Forward Mutation Assay in Bacteria												
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI:</td> <td style="width: 33%;">C. Pueyo</td> <td style="width: 33%;">Guest Worker</td> <td style="width: 10%;">LEM</td> <td style="width: 10%;">NIEHS</td> </tr> <tr> <td>Other:</td> <td>J. Lopez-Barea</td> <td>Visiting Associate</td> <td>LEM</td> <td>NIEHS</td> </tr> </table>			PI:	C. Pueyo	Guest Worker	LEM	NIEHS	Other:	J. Lopez-Barea	Visiting Associate	LEM	NIEHS
PI:	C. Pueyo	Guest Worker	LEM	NIEHS								
Other:	J. Lopez-Barea	Visiting Associate	LEM	NIEHS								
COOPERATING UNITS (if any) None												
LAB/BRANCH Laboratory of Environmental Mutagenesis												
SECTION												
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709												
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:										
1.0	1.0	0										
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS												
SUMMARY OF WORK (200 words or less - underline keywords) A forward mutation assay has been developed in <u>Salmonella typhimurium</u> . The L-arabinose-sensitive strain SV3 of <u>Salmonella typhimurium</u> is being calibrated against mutagens causing base-pair changes, frameshift mutations, and deletions. New strains lacking the excision-repair system and/or the lipopolysaccharide barrier were selected from SV3. The sensitivities of the new strains have been compared with strain SV3. Physiological and biochemical studies will be performed with the strains in order to characterize the <u>L-arabinose-resistance mutagen assay</u> .												

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: Strain SV3 is sensitive to mutagens causing base-pair changes, frameshift mutations and deletions. Three new strains have been selected from strain SV3 which are deficient for the excision-repair system, the lipopolysaccharide barrier, or both. These additional mutations do not affect the assay's freedom from experimental artifacts, and they produce increased sensitivity to certain chemicals. Strain SV3 shows high inducible levels of L-arabinose isomerase and kinase but lacks epimerase, the third enzyme of the L-arabinose operon. The L-arabinose-resistant mutants selected from SV3 have additional mutations in the other genes involved in the L-arabinose pathway.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: At this time, the bacterial strains most widely used for mutagen testing indicate mutagenicity by reversion to wild type. This necessitates the use of a number of specific strains which are capable of responding to different types of genetic effects - base-pair substitutions, frameshifts, and small deletions. This new L-arabinose-sensitive *Salmonella* is capable of responding to mutagens inducing either base-pair substitution, frameshifts or deletions, and therefore may be a valuable adjunct to mutagen testing programs.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60068-01 LEM												
PERIOD COVERED October 1, 1977 to September 30, 1978														
TITLE OF PROJECT (80 characters or less) Evaluation of Metabolic Activation Systems in <u>Salmonella</u>														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT														
PI: Others:	C. Pueyo D. Frezza E. Zeiger B. Smith	<table style="width: 100%; border: none;"> <tr> <td style="width: 60%;">Guest Worker</td> <td style="width: 20%;">LEM</td> <td style="width: 20%;">NIEHS</td> </tr> <tr> <td>Visiting Fellow</td> <td>LEM</td> <td>NIEHS</td> </tr> <tr> <td>Research Microbiologist</td> <td>LEM</td> <td>NIEHS</td> </tr> <tr> <td>Staff Fellow</td> <td>LP</td> <td>NIEHS</td> </tr> </table>	Guest Worker	LEM	NIEHS	Visiting Fellow	LEM	NIEHS	Research Microbiologist	LEM	NIEHS	Staff Fellow	LP	NIEHS
Guest Worker	LEM	NIEHS												
Visiting Fellow	LEM	NIEHS												
Research Microbiologist	LEM	NIEHS												
Staff Fellow	LP	NIEHS												
COOPERATING UNITS (if any) Laboratory of Pharmacology, NIEHS														
LAB/BRANCH Laboratory of Environmental Mutagenesis														
SECTION														
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709														
TOTAL MANYEARS: 0.35	PROFESSIONAL: 0.35	OTHER: 0												
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords) Three metabolic activation systems, <u>intrasanguinous-host mediated assay</u> , <u>isolated perfused organs</u> , and <u>in vitro microsomes</u> are being compared in their effectiveness for detecting <u>chemical mutagens</u> . <u>Dimethylnitrosamine (DMN)</u> was used as the test mutagen and <u>strain SV3 of Salmonella</u> , which detects <u>forward mutations</u> in the <u>arabinose operon</u> , is used as the tester microorganism. <u>CD₁ male mice</u> and <u>CD male rats</u> are used for mammalian activation.														

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: The arabinose-resistance forward mutagen assay in strain SV3 is remarkably well adapted to detect mutagens in body fluids and mammalian tissues. In the host-mediated assay, the bacteria are recovered from lungs, liver, kidneys and blood in proportions higher than has been reported with other bacterial systems. When DMN is used as the mutagen a higher mutagenic response is found with mice compared with rats, with liver and kidneys compared with lungs, and in vivo compared with in vitro microsomal activation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Forward mutation assays are valuable tools for mutagen testing programs since they are theoretically capable of responding to a broad range of mutagens. This new, arabinose-sensitive strain is capable of being used in a variety of in vivo and in vitro metabolic assay systems and therefore can be a useful new system for assaying chemicals for mutagenicity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60069-01 LEM
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Metabolic Activation by S-9 Fractions from Liver Homogenates of Induced Rats		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Other:	T.-M Ong E. Zeiger C. R. Wolf H. Mukhtar	Geneticist Research Microbiologist Visiting Fellow Visiting Fellow LEM NIEHS LEM NIEHS LET NIEHS LP NIEHS
COOPERATING UNITS (if any) Laboratory of Environmental Toxicology, NIEHS Laboratory of Pharmacology, NIEHS		
LAB/BRANCH Laboratory of Environmental Mutagenesis		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) 9000 x g supernatant (S-9) of <u>liver homogenates</u> from mammals is commonly used in the <u>in vitro</u> activation system to study the <u>mutagenicity</u> of <u>chemical carcinogens</u> which require metabolic activation for their biological activities. Several studies have shown that the mutagenic activity of these compounds can be enhanced if S-9 is from animals pretreated with enzyme inducers such as <u>phenobarbital</u> (PB), <u>3-methylcholanthrene</u> (3-MC), or <u>Arochlor 1254</u> . Among these inducers, Arochlor 1254 appears to be less specific and can enhance a variety of enzyme activities. It has been shown, however, that Arochlor is carcinogenic. Studies using the <u>Salmonella</u> plate incorporation test system strains TA98 and TA1535 are being conducted to determine whether combined injection of <u>β-naphthoflavone</u> and PB can be used as a safe substitute for Arochlor as inducers in the <u>in vitro</u> activation system.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Seven week old male rats are divided into 6 groups. Each group is pretreated either with phenobarbital (PB), β -naphthoflavone (NF), Arochlor 1254 or a combination of PB and NF. Protein and cytochrome-P-450 contents, and the activities of aryl hydrocarbon hydroxylase, benzphetamine N-demethylase, epoxide hydrolase and glutathione S-transferase are determined in S-9 of liver homogenates from these animals. Metabolic activation of 2-anthramine, 2-acetyl-aminofluorene, 3-methylcholanthrene and benzo(a)pyrene to mutagenic metabolites by S-9 from rats pretreated with different inducers are also studied with TA1535 or TA98 of Salmonella typhimurium.

MAJOR FINDINGS AND PROPOSED COURSE: Major findings include: (1) enzyme activities of S-9 do not correlate with mutagenic responses, (2) different compounds have different mutagenic responses to the same inducer, (3) the mutagenic response of a given compound to different inducers appears to depend upon concentrations of the compound tested, and (4) although Arochlor appears to be a better inducer, similar patterns of mutagenic response are obtained with different chemicals if S-9 from Arochlor-pretreated, or a combination of NF- and PB-pretreated, animals are used. More compounds need to be studied in the future to determine whether a combination of NF and PB can be used as substitutes for Arochlor.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Arochlor is commonly used as an enzyme inducer both in pharmacology and mutagenesis. This mixture is now known to be carcinogenic. Seeking safe substitutes for Arochlor in the mutagenesis testing program is therefore important to the safety of the personnel who are working with enzyme inducers.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60070-01 LEM
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Mutagenic Activity of <u>Penicillium roqueforti</u> Toxin in Microbial Test Systems		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Other:	R. Wei W. Whong D. Frezza E. Zeiger	Visiting Scientist Visiting Fellow Visiting Fellow Research Microbiologist
		LEM NIEHS LEM NIEHS LEM NIEHS LEM NIEHS
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Environmental Mutagenesis		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 1.2	PROFESSIONAL: 1.2	OTHER: 0
CHECK APPROPRIATE BOX(ES)		
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER		
☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) <u>PR toxin</u> is a <u>mycotoxin</u> isolated from <u>Penicillium roqueforti</u>, a fungus used in the ripening of roquefort and blue cheeses. Previous studies have shown that PR toxin can inhibit biosynthesis of protein and nucleic acids in mammalian tissues. A preliminary study by J. A. Miller at the University of Wisconsin indicates that PR toxin induces tumors in rats. Chemically, PR toxin has a bis-epoxide functionality, which is ordinarily considered to be highly reactive and to display mutagenic effects. The purpose of this work is to determine the <u>mutagenicity</u> of PR toxin in different microbial test systems. <u>Saccharomyces cerevisiae</u>, <u>Neurospora crassa</u> and <u>Salmonella typhimurium</u> are employed in a liquid suspension procedure. The effects of different pH and absence or presence of liver S-9 enzymes were also examined.		

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: (1) PR toxin at concentrations of 1 to 10 $\mu\text{g/ml}$ was toxic to Saccharomyces cerevisiae, Neurospora crassa and Salmonella typhimurium. The survival rate was decreased by changing pH from acid and neutral to alkaline. (2) The toxin at a concentration of 2.5 $\mu\text{g/ml}$ increased the Trp⁺ and Ade⁺ revertant frequency of the Saccharomyces cerevisiae D₄ strain by four- and two-fold, respectively. (3) In the presence of liver S-9 enzymes in the test solution, the mutagenic activity of the toxin vanished. These preliminary results indicate that PR toxin itself is a mutagen. Further study on Saccharomyces cerevisiae strain D₇, Neurospora crassa and Salmonella typhimurium are being conducted. In addition, the metabolic fate of the toxin will also be examined in these test organisms and with liver S-9 fraction.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

P. roqueforti is used for roquefort and blue cheese production. Although the possibility of this toxin being present in cheese is not known, from a chemical point of view it is of interest to evaluate it for its potential to induce heritable genetic alterations. The data obtained from this program will be useful for determining the safety of this bis-epoxide compound and for understanding its biological activity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60071-01 LEM
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Activation of Carcinogens to Mutagens in <u>Salmonella</u> by Rat Hepatocytes		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: T.-M. Ong Other: J. Guthrie E. Zeiger	Geneticist Microbiologist Research Microbiologist	LEM NIEHS LEM NIEHS LEM NIEHS
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Environmental Mutagenesis		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.1	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Some chemical carcinogens which can be activated to metabolites mutagenic to <u>Salmonella typhimurium</u> by 9000 Xg supernatant of liver homogenates from different mammalian species are carcinogenic only in certain species. Some other chemical carcinogens are not mutagenic in <u>S. typhimurium</u> with or without in vitro S-9 activation systems. Studies using <u>S. typhimurium</u> tester strains. TA98 and/or TA1535 are conducted to determine whether those false-negative compounds in bacteria mutagenesis test systems can be activated to mutagenic metabolites by hepatocytes from these species. Bacterial cells are treated with carcinogens and hepatocytes in suspension for 2 to 24 hrs. After treatment bacteria are washed and plated both in minimal media and minimal medium supplemented with histidine. Revertants and survival colonies are scored after 2 days incubation at 37°C.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Hepatocytes are isolated from liver of 4 to 6 weeks old female rats by a collagenase/hyaluronidase technique. Cells (1×10^9) of Salmonella typhimurium strain TA98 are treated with Benzo(a)pyrene (BP) or 2-acetylaminofluorene (2AAF) and 4×10^6 hepatocytes in 3 ml L15 medium. After 2 to 24 hrs treatment, bacterial cells are washed by centrifugation. Appropriate numbers of cells are then plated both in minimal medium and minimal medium supplemented with histidine. Revertants and survival colonies are scored after 2 days incubation at 37°C.

MAJOR FINDINGS AND PROPOSED COURSE: Conclusive results are not yet available. The preliminary studies indicate that rat hepatocytes can activate BP and 2AAF to metabolites mutagenic to TA98 of S. typhimurium. A standard protocol needs to be defined in future studies. Other chemical carcinogens and hepatocytes from other animal species should also be used in the future work.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Application of hepatocytes in mutagenesis assays could provide important information in regard to the mutagenicity of those false-negative compounds in bacterial assay systems, specifically as to why some chemical carcinogens are carcinogenic only in certain animal species.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60072-01 LEM
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Genetic Duplications in Salmonella		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: G. R. Hoffmann Geneticist LEM NIEHS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Environmental Mutagenesis		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.3	PROFESSIONAL: 0.3	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (a1) MINORS ☐ (a2) INTERVIEWS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER		
SUMMARY OF WORK (200 words or less - underline keywords) A selection procedure has been developed for the detection of a large <u>genetic duplication</u> in <u>Salmonella typhimurium</u> . It has been found that a variety of <u>mutagens</u> increase the rate of duplication formation. Mutagens also increase the rate of loss of duplications from merodiploid strains.		

PROJECT DESCRIPTION

METHODS EMPLOYED: The procedures used in this project are standard methods of microbial genetics including conjugation, transduction, selection and characterization of mutants and duplications, chemical mutagenesis, and irradiation with ultraviolet light and X-rays.

MAJOR FINDINGS AND PROPOSED COURSE: A genetic duplication involving approximately 30% of the Salmonella typhimurium chromosome can be obtained by selecting for increased rates of growth on L-malate as the sole carbon source. The spontaneous duplication frequently is approximately 10^{-4} per cell plated. Following treatment with mutagens there are dose-dependent increases in duplication frequency above the spontaneous level. The duplication is genetically unstable, and haploid segregants are obtained at high frequency in merodiploid strains. In addition to inducing duplications in haploid strains, mutagens have been found to stimulate the rate of duplication-loss from merodiploids. Experiments now in progress are designed to provide stronger selection conditions for genetic duplications. In addition, studies now being initiated will provide information regarding mechanisms of duplication formation and loss.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In addition to inducing point mutation, mutagenic agents are known to have a number of other genetic effects on biological systems including the induction of chromosome aberrations, mitotic crossing over, mitotic gene conversion and sister chromatid exchange. This project involves the characterization of yet another effect of mutagens -- the induction of duplication formation and loss in bacteria. A fuller understanding of the biological activity of mutagenic agents requires study of the full spectrum of genetic consequences attributable to them. It is quite possible that the effects studied in this project are a manifestation of the recombinogenicity of these agents. Present studies are providing information on this possibility. In view of the fact that the correlation between mutagenicity and carcinogenicity appears to extend to recombinogenicity as well, development of model systems for the study of recombinogenic effects of mutagens is of both theoretical and practical significance.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60073-01 LEM
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Mutagen Specificity in <i>Saccharomyces cerevisiae</i>		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Other:	R. C. Harvey G. R. Hoffmann F. J. de Serres	Geneticist Geneticist Assoc. Dir. for Genetics
		LEM NIEHS LEM NIEHS OD NIEHS
COOPERATING UNITS (if any) Office of the Director, NIEHS		
LAB/BRANCH Laboratory of Environmental Mutagenesis		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 1.3	PROFESSIONAL: 0.3	OTHER: 1.0
CHECK APPROPRIATE BOX(ES)		
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER		
☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) A genetic system is being developed in the yeast <i>Saccharomyces cerevisiae</i> for the simultaneous study of <u>chemical mutagen specificity</u> by a variety of approaches. Other <u>forward mutation</u> and <u>reverse mutation</u> assays in fungal test systems have yielded data that are contradictory with regard to the predominating classes of molecular alterations induced by specific chemical mutagens. This system is designed to minimize differential effects of extrinsic and physiologic factors that influence mutagen specificity.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Tetrad dissection and random spore isolation are used in the construction of multiply marked isogenic strains of S. cerevisiae. Forward mutations at the ade loci will be detected by screening colonies for pigment accumulation. Reverse mutation will be detected by the growth of revertants on selective culture media. Genetic characterization of induced mutants will provide data on the specificity of the chemical mutagen for preferentially inducing various classes of molecular alterations in the DNA.

MAJOR FINDINGS AND PROPOSED COURSE: This project is in the preliminary stages of development.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Quantitative data on the mutagenicity of chemical compounds is being rapidly accumulated, but there is much less information available on the qualitative aspects of mutagenesis. Discrepancies in the existing literature show that our understanding of chemical mutagen specificity is as yet rather primitive. A difficulty in studying mutagen specificity is that factors other than the interaction of the mutagen with DNA can alter specificity. It is hoped that this project will result in the development of strong methods of genetic analysis for mutagenicity studies and will contribute significantly to an increased understanding of both chemical mutagen specificity and the profound differences sometimes observed with different assay systems.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60074-01 LEM						
PERIOD COVERED October 1, 1977 to September 30, 1978								
TITLE OF PROJECT (80 characters or less) Genetic Effects of Selected Substances in Yeast								
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: G. Bronzetti</td> <td style="width: 33%;">Visiting Associate</td> <td style="width: 34%; text-align: right;">LEM NIEHS</td> </tr> <tr> <td>Other: E. Zeiger</td> <td>Research Microbiologist</td> <td style="text-align: right;">LEM NIEHS</td> </tr> </table>			PI: G. Bronzetti	Visiting Associate	LEM NIEHS	Other: E. Zeiger	Research Microbiologist	LEM NIEHS
PI: G. Bronzetti	Visiting Associate	LEM NIEHS						
Other: E. Zeiger	Research Microbiologist	LEM NIEHS						
COOPERATING UNITS (if any) None								
LAB/BRANCH Laboratory of Environmental Mutagenesis								
SECTION								
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709								
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0						
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS								
SUMMARY OF WORK (200 words or less - underline keywords) A number of chemicals of interest are being tested for <u>mutagenicity</u> and <u>recombinogenicity</u> in <u>Saccharomyces cerevisiae</u> D7. One purpose of the study is to evaluate substances which are genetically active in yeast but not in <u>Salmonella</u>. Another, is to compare the activity of yeast with <u>Salmonella</u> for chemicals which are known to be active in <u>Salmonella</u>. Among the chemicals which will be studied are <u>procarbazine</u> (mutagenic in yeast but not in <u>Salmonella</u>), <u>Benzo(a)pyrene</u>, and <u>2-aminoanthracene</u>.								

PROJECT DESCRIPTION

METHODS EMPLOYED: Yeast and bacteria will be exposed to the test chemicals in suspension with and without a 9000 Xg mouse liver preparation. Mutants and recombinants will be evaluated using standard techniques.

MAJOR FINDINGS AND PROPOSED COURSE: Procarbazine, an antitumor agent, is an effective mutagen in Saccharomyces D7 but is not mutagenic for Salmonella. Studies with the other substances are in progress or are yet to be initiated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: As opposed to Salmonella, Saccharomyces is a eukaryotic microorganism in which it is possible to measure mitotic effects - such as gene conversion and recombination - in addition to mutation. There has been very little systematic work done with yeast using mutagen assays compared with bacteria. This study will attempt to evaluate the suitability of yeast for mutagen assays with regard to range of chemical classes to which it will respond and dose sensitivity as compared to a bacterial system. This will determine the future role of yeast in mutagen screening programs.

PROJECT DESCRIPTION

METHODS EMPLOYED: In the intrasanguineous host-mediated assay, the yeast were injected into the tail vein of Cd-1 mice and the test substances administered by gavage. After a fixed exposure time, the mice were sacrificed, the yeast recovered from the liver, lungs and kidneys and examined for mutation, mitotic gene conversion and recombination. In the in vitro assay the yeast was exposed to the test chemicals in flasks either in buffer or in the presence of a 9000 Xg mouse liver preparation.

MAJOR FINDINGS AND PROPOSED COURSE: Trichloroethylene has been completed. There is a significant, reproducible genetic effect in vitro using mouse liver activation. In the host-mediated assay, the strongest effect is seen in yeast recovered from the liver, followed by the kidneys. Virtually no effect is seen in the lungs (TCE is a strong liver carcinogen). Studies with dichloroethylene, tetrachloroethylene and TCDD are currently in progress.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Chlorinated hydrocarbons are widely used industrially and are produced in large quantities. Recent evidence has shown them to be carcinogenic. In bacterial test systems they show up as weakly mutagenic, usually with modified test protocols. In this and other studies Saccharomyces has shown itself to be an excellent test organism for these substances. It is expected that using this and other studies we will be able to demonstrate the relationship between chemical structure and genetic activity in yeast. Also, we are attempting to see whether the organ specificity in the host-mediated assay is predictive of carcinogenic organ specificity.

PUBLICATIONS

Bronzetti, G., Zeiger, E., and Frezza, D.: Genetic effects of trichloroethylene in yeast. J. Environ. Pathol. Toxicol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60076-01 LEM
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Genetic Effects of 1,2,7,8-Diepoxyoctane in Chinese Hamster Lung Cells		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: S. L. Huang Senior Staff Fellow LEM NIEHS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Environmental Mutagenesis		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 1	PROFESSIONAL: 1	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The <u>mutagenicity of 1,2,7,8-diepoxyoctane (DEO)</u> was investigated using <u>Chinese hamster lung cells</u> . <u>6-thioguanine resistance and chromosome aberration</u> were induced. DEO was found to readily form <u>adducts with radiolabeled nucleotides</u> , as demonstrated through <u>thin-layer chromatography</u> in DEO/nucleotides mixtures. The mutagenic specificity of DEO is apparently related to the compound's ability to bind DNA.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Two million cells were treated with DEO for one hour. The treated cells were dispersed and 10^5 cells were inoculated in petri dishes. Selection for mutants was initiated after a two-day expression time. Chromosome preparation was done according to Ford and Hamerton's method. Radionucleotides were incubated in phosphate buffer with DEO. Adducts were separated by thin-layer chromatography on MN 300 cellulose plates, using an ethanol and ammonium acetate solvent system.

MAJOR FINDINGS AND PROPOSED COURSE: DEO was mutagenic to animal cells. A dose-response relationship was demonstrated for induced TG resistance and chromosome aberrations using DEO. All the TG^r colonies obtained were HGPRTase-deficient and the resistant phenotype was stable.

Epoxides have been identified as the probable ultimate carcinogenic form of many environmental agents. Studies of mono- and bifunctional epoxides have demonstrated their frequent mutagenicity and carcinogenicity in *N. crassa* and *D. melanogaster*. The purpose of this study is to evaluate and compare the usefulness and reliability of presently available mutation test systems in cultured mammalian cells. This evaluation would aid in the comparison of results from several test systems and in the extrapolation to human studies. Prior experiments indicated that the amount of bound carcinogens or mutagens correlates with the degree of induced mutations in cultured cells. In order to understand the mechanisms of mutation, experiments to study the binding of DEO with nucleotides were also carried out.

New genetic systems using cultured mammalian cells are being developed and the mechanisms that are responsible for the genetic lesion will be studied.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The Lesch-Nyhan syndrome is a hereditary disorder of prime metabolism in man. The responsible gene is a sex-linked recessive. Chromosome aberrations are also known to cause human genetic defects. A mutagen test system using this locus and also scoring chromosome aberrations can be employed to monitor the induction of mutations by environmental agents.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60077-01 LEM
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Molecular Mechanisms of Heat-Induced Mutation		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: L. S. Ripley Senior Staff Fellow LEM NIEHS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Environmental Mutagenesis		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Heat-induced mutations in bacteriophage T4 have been demonstrated to occur by at least two distinct mechanisms: (1) G:C base pairs are converted to A:T base-pairs (<u>transition</u>), (2) G:C base-pairs are converted to T:A or C:G base-pairs (<u>transversion</u>). The C of the base-pair is the target of the first mechanism. The G of the base-pair is the target of the second mechanism. Studies of the step(s) required for conversion of the heat-induced lesion (pre-mutational lesion) to the fully expressed mutation are continuing. The specificity of the transversion reaction (G:C to T:A versus G:C to C:G) is to be determined. T4 phage contain glucosylated hydroxymethylcytosine in their DNA. The influence of this modification on mechanism (1) will be explored.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard genetic manipulations in bacteriophage T4 are used. Mutations are measured in the rII genes. Appropriate mutants are available for making T4 with DNA which is non-glucosylated or which has C rather than hydroxymethyl-C. To distinguish G:C to T:A transversions from G:C to C:G transversions, a missense mutant having a serine (UCA) or a tyrosine (UAC) codon is needed.

MAJOR FINDINGS AND PROPOSED COURSE: Work to date, on the basis of statistical analysis of mutations arising in single bursts, indicates that heat modified C-sites are converted to mutations less than 50% of the time when they are replicated. The suspected premutational lesion is glucosylated hydroxymethyl-uridine, arising as a result of the deamination of the modified cytosine in the DNA. This lesion would be predicted to give rise to mutation at a higher frequency than 50% if hydrogen bonding properties of a base solely predicted its probability of pairing during replication. No repair pathways have been demonstrated. Recombinationally arising heteroduplex heterozygotes having G paired with A or C paired with T give rise to equal numbers of progeny from both DNA strands.

A study of the effect of glucosylation or 5-hydroxymethylation of C on mispairing properties of heat-treated cytosine will perhaps give an insight as to possible mechanisms used during replication to distinguish heat-modified cytosine from a better analogue of thymine.

Indirect evidence suggests that heat induced transversion mutations include G:C to C:G transversions, but no evidence is available whether G:C transversions to T:A are also induced. Heat-induced mutation is the only modification of a particular base at present known to induce transversion mutations specifically. A further understanding of the reaction could allow better insight into the mechanism of this reaction. Measurement of heat-induced reversion from a UCA codon would allow direct measurement of the two transversion types by measuring the relative frequency of revertants to UGA (opal) and UAA (ochre). No UCA or UAC codons amenable to heat-induced transversions have yet been identified.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Heat is a ubiquitous mutagen in the environment. All organisms must have mechanisms to repair the heat-induced pre-mutational lesions or else sustain a rather high level of mutation proportional to the number of G:C base-pairs in their DNA. For organisms with large genomes this may represent a serious challenge. T4 is relatively amenable to experimental exposure to heat mutagenesis, and is at least partially insensitive to repair mechanisms evolved by its host, E. coli, for dealing with pre-mutational lesions. This allows the study of mutagenic mechanisms uncomplicated by repair processes. A more detailed study may uncover new mechanisms for dealing with pre-mutational lesions by the replication machinery itself and lead to new insights into accuracy determination in the replication of DNA.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60078-01 LEM
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) T4 DNA Polymerase (Gene 43) Error Rate with DNA Containing 2-Aminopurine		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: L. S. Ripley Senior Staff Fellow LEM NIEHS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Environmental Mutagenesis		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (e1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) This is a study of the error rates of mutator, anti-mutator and wild type T4 DNA polymerase (gene 43) in 2-aminopurine-substituted DNA in vivo. Base analogue-induced mutation rates can be influenced both by the degree of incorporation of the analogue into the DNA and by the frequency of errors of replication past incorporated 2-aminopurine. An appropriate method is being sought to separate the replication from the incorporation step of mutagenesis by 2-aminopurine. Preliminary evidence suggests that separation of replication and incorporation may be achieved by mixed infection between one phage substituted with 2-aminopurine carrying one allele of gene 43, and a second phage which supplies a second allele (mutator, anti-mutator or wild type) of gene 43. Quantitative analysis of mutagenesis in these mixed infections may allow determination of error rates <u>in vivo</u> for different gene 43 alleles independent of incorporation rates.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard genetic manipulations in bacteriophage T4 utilizing the well-characterized mutants of the rII genes to measure mutation rates and well-characterized alleles of T4 DNA polymerase which have been shown in vivo and in vitro to have altered fidelity of DNA replication.

MAJOR FINDINGS AND PROPOSED COURSE: Preliminary findings suggest that separation of replication and incorporation may be achieved by using a phage stock grown in the presence of 2-aminopurine, having a biochemically defined average frequency of 2-aminopurine residues in its DNA and a genetically defined frequency of mutation at particular sites. This phage stock can be further grown in the absence of additional 2-aminopurine but in the presence of different alleles of gene 43 polymerase. Different alleles of gene 43 can be added by mixedly infecting a single bacterium with DNA of two different phages. One phage carries the rII mutant whose reversion frequency is being measured and is the phage pre-treated with 2-aminopurine. The second phage carries a deletion of the rII region and therefore does not contribute to the reversion of the rII mutant, but does carry an additional gene 43 allele which might then act on the 2-aminopurine incorporated at the mutant site in the first phage.

Experiments of Goodman *et al.* (PNAS 74:4806-4810) have shown that average incorporation of 2-aminopurine in packaged T4 DNA by a mutator allele of gene 43, ts L56, is not significantly higher than incorporation by the wild-type allele, and that incorporation by the anti-mutator allele of gene 43, ts L141, is only about 5-fold lower than incorporation by the wild-type allele (in agreement with in vitro measurements). However, differences in the mutation rates at certain genetic sites in the presence of these different alleles of gene 43 may differ by 4 orders of magnitude. This suggests that primary differences in mutation rates can be attributed to replication error rates.

This experiment may allow a direct measurement of the replication error rates of these polymerases. The simple prediction, that phage grown in the presence of 2-aminopurine in the presence of the anti-mutator allele should give enhanced mutagenesis when grown further in the presence of the wild-type or mutator allele, relative to phage not treated with 2-aminopurine or to phage grown further in the presence of only the anti-mutator allele, has been fulfilled.

Spontaneous mutation rates are influenced by these alleles of gene 43 and the influence on spontaneous mutation frequency can be used to measure the trans-acting frequency of replication (via mutation) in the mixed infections. If the trans-action is not strongly affected by the presence of 2-aminopurine in the DNA, a measurement of the rate of mutagenesis due to replication past incorporated 2-aminopurine in the DNA can be measured.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: An understanding of the molecular mechanisms by which the DNA replication machinery regulates the level of mutation in an organism is necessary for a

basic understanding of mutation, inherent error rates in DNA replication, and errors of replication due to the impact of the environment upon the organism. This study adds to our knowledge of how a DNA polymerase distinguishes aberrant DNA constituents.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60079-01 LEM
PERIOD COVERED November 7, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Mutants Show Mutational Synergy with the Replicative DNA Polymerase of <u>E. coli</u>		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT P.I. E. B. Konrad Senior Staff Fellow LEM NIEHS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Environmental Mutagenesis		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 1	PROFESSIONAL: 1	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) E. coli normally replicates its DNA with a very high degree of fidelity. It is not known whether this is achieved by the <u>replicative DNA polymerase</u> (polymerase III) alone, by the polymerase acting in concert with other proteins, or by the polymerase together with post-replication repair systems. One approach to sorting out this problem is to take a strain with a mutationally altered polymerase III which has been rendered mutagenic and look for mutations in the strain which show <u>mutational synergy</u> , that is, mutations which are strongly mutagenic when in the same strain with a polymerase III mutation, but show relatively little mutagenicity by themselves. Such mutants must interact directly or indirectly with polymerase III. Using a novel <u>papillation</u> technique a number of mutations which show a high degree of synergy with polymerase III have been isolated. Biochemical and genetic techniques will be used to determine whether these mutations are alleles of mutations known to affect DNA metabolism, thus suggesting a role in maintaining replicative fidelity for the corresponding wild-type protein, or are novel mutants affecting post-replication DNA repair.		

PROJECT DESCRIPTION

METHODS EMPLOYED: I have developed a simple method for estimating relative frequencies of mutation in single colonies of Escherichia coli. This method consists of plating single colonies of a strain of E. coli, lactose-negative due to a point mutation in the gene encoding β -galactosidase (lacZ), on the lactose-indicator plate lactose-tetrazolium. On this indicator plate, lactose-negative colonies are red, while lactose-positive colonies are white. When the lactose-negative strain is plated on lactose-tetrazolium, it initially forms red lactose-negative colonies. However, after prolonged incubation, white, lactose-positive papillae form on the surface of these colonies. Each of the lactose-positive papillae represents a clone descended from a lactose-positive revertant. The number of papillae on each colony is proportional to the mutation frequency of the cells within the colony.

MAJOR FINDINGS AND PROPOSED COURSE: DNA polymerase III is the polymerase responsible for DNA replication in E. coli. A number of temperature-sensitive conditional lethal mutations have been isolated in the structural gene for this enzyme (polC). At least some of these mutations are also mutator mutations. Using my papillation test for relative mutagenicity, I have isolated a number of mutations which interact synergistically with polC with respect to mutation frequency; that is, mutations which confer a high frequency of mutation on strain carrying polC, but have little or no effect on the mutation frequency of strains not carrying polC. Synergy strongly implies that the products of these mutant genes interact directly or indirectly with polymerase III in maintaining replicative fidelity. These mutations are currently being mapped to determine whether they correspond to mutations already known to play a role in DNA replication. Those that do not will be tested in vitro for their capacity to affect mismatch repair in DNA heteroduplexes.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It is not yet understood how replicative fidelity is achieved. The number of loci known to effect DNA replication or mutation frequencies suggests that this fidelity may be attained through the interactions of several proteins. Those that interact with DNA polymerase III to promote fidelity should be revealed in this project. An increased knowledge of the mechanism of DNA replication should be valuable in understanding environmental mutagenesis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60080-01 LEM
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Modification of Site-Specific Mutation Rates by a Nearby Base-Pair Substitution		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: M. A. Conkling Geneticist LEM NIEHS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Environmental Mutagenesis		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Some evidence supports the hypothesis that the <u>mutation rate</u> at a particular site is influenced by <u>neighboring base pairs</u> . I have sought to modify the <u>reversion</u> and <u>conversion frequencies</u> of <u>rII nonsense</u> codons by inserting nearby <u>temperature-sensitive rII lesions</u> . The <u>spontaneous</u> and <u>2-aminopurine induced</u> reversion rate of an <u>amber mutation</u> is decreased threefold by inserting a <u>ts</u> mutation 10 to 30 base pairs away. Site-specific mutation rates will be measured using the reversion and conversion pathways of the corresponding ochre mutation.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Double rII mutants have been constructed composed of one of two homologous nonsense mutants (rHB129am and rHB129oc) plus a ts mutation (rPS78). The spontaneous and 2-aminopurine induced reversion and conversion frequencies of the nonsense mutations are measured in the presence or absence of the ts mutation.

MAJOR FINDINGS AND PROPOSED COURSE: The spontaneous and 2-aminopurine induced reversion frequencies of rHB129am is reduced about threefold in the presence of rPS78. I can demonstrate that the modified reversion frequency does not appear to reflect an altered efficiency of detection of revertants, but rather an alteration in the mutation rate itself. Preliminary data suggest that the spontaneous and 2-aminopurine induced reversion of rHB129oc is not affected by the ts mutation. The 2-aminopurine induced conversion of rHB129oc to rHB129am is reduced approximately threefold in the presence of the ts mutation. The spontaneous conversion frequencies of rHB129oc to rHB129op and to rHB129am remain to be determined.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The characteristic mutation rate of a genetic site is often supposed to depend not only upon the base-pair at that site, but also upon the molecular environment determined by nearby base-pairs. This idea seems to have originated as an explanation of mutational "hot spots." A rigorous demonstration of a neighboring base effect requires that a mutation rate be altered as a result of a nearby base-pair substitution. An understanding of the fundamental mechanisms of mutagenesis requires an understanding of the role of the molecular environment upon mutation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60081-01 LEM
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) The Genetics of the Misrepair Mutagenesis System of Bacteriophage T4		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: M. A. Conkling Geneticist LEM NIEHS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Environmental Mutagenesis		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Lesions induced in DNA may be repaired by "error-proof" or "error-prone" repair mechanisms. Error-prone systems repair the lethal damage of the lesion, but in doing so may fail to restore the original nucleotide sequence, producing mutations. This error-prone process is called misrepair mutagenesis. Bacteriophage T4 has its own misrepair pathway, mediated by at least three genes, <u>x</u> , <u>y</u> , and <u>w</u> . Mutations in two of these genes, <u>x</u> and <u>y</u> , suppress mutations of gene 49, a gene involved in DNA packaging. This allows the selection of alleles of <u>x</u> and <u>y</u> . Amber and <u>ts x</u> and <u>y</u> mutants are being sought. These will allow the time course of mutagenesis to be examined. An <u>rII</u> reversion system to assay misrepair mutagenesis is being developed that will increase the sensitivity and allow measurements of mutagenesis along certain defined pathways. Finally the enzymology of T4 misrepair mutagenesis will be studied by genetic and biochemical approaches.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Suppressors of gene 49 amber mutations will be selected. These can be arranged into complementation groups using a complementation test based on UV sensitivity. Their sensitivities to inactivation and mutagenesis by UV irradiation will be determined.

MAJOR FINDINGS AND PROPOSED COURSE: Mutations in genes involved in T4 misrepair mutagenesis result in decreased recombination frequencies, increased sensitivity to inactivation by UV or gamma irradiation, and decreased sensitivity to mutagenesis by UV or gamma irradiation. Mutations in genes x and y can suppress amber and ts mutations of gene 49, a gene involved in DNA packaging. Amber and ts x and y mutants are being sought. These will allow temperature-shift experiments to study the time course of misrepair mutagenesis. The enzymology of T4 misrepair mutagenesis will be studied by genetic and biochemical approaches. The genetic approach will look at interactions of genes involved in DNA metabolism in the misrepair system. The biochemical approach will involve an in vitro system to measure misrepair.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Although the misrepair pathway is thought to be a major pathway of mutagenesis in eucaryotic systems, the biochemistry of this repair process is not understood. Bacteriophage T4 offers a simple model system to begin to understand the enzymology of the misrepair process and also offers a highly defined genetic system to study mutagenesis along certain defined pathways. Alleles of genes involved in T4 misrepair can easily be selected. In vitro systems for T4 replication have been developed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60082-01 LEM
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Neoguanosine Pairing During DNA Synthesis		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: D. C. Mace Staff Fellow LEM NIEHS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Environmental Mutagenesis		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) This project will investigate the possibility that <u>deoxyneoguanosine</u> residues in DNA (hypothesized to be the <u>GC+CG transversion pathway intermediate</u> for heat mutagenesis in T4 bacteriophage; Bingham et al., PNAS (1976), 73:4159-4163 can indeed form a base pair with a deoxyguanosine residue on the opposing strand of a DNA double helix. Upon completion of the synthesis of <u>deoxyneoguanosine triphosphate</u> (dNGTP; now being carried out at the University of Illinois) the possible utilization of this compound as a substrate for <u>in vitro</u> DNA polymerization will be tested using T4 DNA polymerase and E. coli DNA polymerase I. If there is an indication of utilization, <u>synthetic polymers</u> will be made containing various natural deoxynucleoside monophosphate residues and various mole fractions of deoxyneoguanosine monophosphate residues. Synthesis will be attempted using terminal deoxynucleotidyl transferase. If these can be obtained they will be used for <u>physiochemical studies on deoxyneoguanosine base pairing</u> and for examination of its <u>templating properties for in vitro DNA synthesis</u> .		

PROJECT DESCRIPTION

METHODS EMPLOYED: Synthetic DNA homopolymers containing various deoxynucleotides, including deoxyneoguanosine, will be prepared by established techniques, as will the analysis of the utilization of the deoxyneoguanosine triphosphate by DNA polymerases.

MAJOR FINDINGS AND PROPOSED COURSE: This work will be initiated upon the completion of the synthesis of deoxyneoguanosine triphosphate, which synthesis is being undertaken by another researcher at the University of Illinois.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The heat-induced GC→CG pathway could be a major contributor to the mutational load sustained by the mammalian genome. Thus, it seems important to determine if the hypothesized transversion pathway (involving a thermally induced migration of the glycosylic bond of deoxyguanosine) actually does give rise to a product that can form a base-pair with guanosine during the synthesis of DNA by T4 DNA polymerase. If the results indicate that deoxyneoguanosine is involved, investigations into the existence of this pathway and its repair will be pursued in mammalian cell systems.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60083-01 LEM
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Interaction of T4 DNA Polymerase and T4 Gene 32 Protein		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: D. C. Mace Staff Fellow LEM NIEHS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Environmental Mutagenesis		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.4	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The goal of this project is to reinvestigate the interaction of T4 DNA polymerase and T4 gene 32 protein. Work by this investigator on the rate of polymerization by the T4 DNA polymerase in vitro lead to a number more than one order of magnitude greater than that previously measured for this polymerase. The previous measurement also claimed this rate to have been increased by the presence of T4 gene 32 protein. However, the stimulated rate was not greater than the subsequently measured rate for the polymerase alone. Since there is a clear contradiction in these results, I will attempt to discover the reason for this contradiction (most probably the result of a significant level of binding of the polymerase to single-stranded DNA as well as primer sites, the former of which is greatly reduced in the presence of gene 32 protein, a single-stranded DNA binding protein). If the formerly claimed stimulation is for such a trivial reason, I will attempt to analyze what direct interaction gene 32 protein does have with its homologous polymerase.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Ordinary radioisotope techniques will be employed for studies of DNA polymerization with and without gene 32 protein. The template and primer will be constructed from a variety of restriction fragments made from λ and T7 bacteriophage DNA's. The extent of synthesis will be monitored at extremely short reaction times by isolating the products of the reactions and subjecting them to restriction nuclease digestion and gel analysis. This should provide a sensitive method for the analysis of both the absolute rate of polymerization and the processive character of the polymerization both in the absence and in the presence of gene 32 protein.

MAJOR FINDINGS AND PROPOSED COURSE: This work has just been initiated and will continue to the end of the fiscal year.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In order to understand the mutational process, it will be necessary to understand not only the mechanism of the polymerization process on the part of a solitary DNA polymerase molecule, but also to understand the way in which other proteins involved in DNA replication interact with the polymerase. This is especially so for the case of protein products of genes with mutator alleles, as is the case for gene 32 protein of bacteriophage T4. Towards this understanding, the question of the interaction of T4 DNA polymerase and T4 gene 32 protein is being reinvestigated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60084-01 LEM
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Fidelity of Templated Polymerizing Enzymes During Polymerization		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: D. C. Mace Staff Fellow LEM NIEHS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Environmental Mutagenesis		
SECTION 		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.4	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (a1) MINORS ☐ (a2) INTERVIEWS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER		
SUMMARY OF WORK (200 words or less - underline keywords) The goal of this project is to understand the mechanisms by which templated nucleotide polymerizing enzymes achieve <u>fidelity</u>. Previous workers have concentrated on the 3'→ 5' exonuclease associated with many DNA polymerases, analyzing the role that this activity plays in proofreading the results of the polymerizing step. However, the mechanism by which the <u>polymerizing step</u> achieves accuracy has been largely ignored. To investigate the latter I intend to examine the effect of polymerase substrate analogues on the fidelity of the polymerization reaction. Nucleotide triphosphates modified in the <u>phosphate moieties</u> (either the β or γ position or both) will be utilized with the view that a decreased rate of polymerization is likely to manifest itself as a decrease in the fidelity of polymerization. Both <u>misincorporations</u> and <u>indirect changes</u> effected by altered fidelity at the polymerization step will be analyzed. (Note that by only altering the β and γ positions, there should be no effect of the modification once the β-γ pyrophosphate bond has been hydrolyzed, e.g. no affect on the 3'→ 5' exonuclease).		

PROJECT DESCRIPTION

METHODS EMPLOYED: The preparation of modified deoxynucleoside triphosphates will be accomplished by techniques already established for ribonucleoside triphosphates. Standard methods will also be employed to characterize their utilization by the T4 DNA polymerase.

MAJOR FINDINGS AND PROPOSED COURSE: This work has only recently been initiated and will continue until the end of the fiscal year.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Understanding the mechanisms used by DNA polymerases to accurately replicate the template during the polymerization step of DNA synthesis has largely been ignored until now. Such experiments are vital to understand how the in vivo replication of DNA can be accomplished with sufficiently few errors to maintain a biologically acceptable spontaneous mutation rate. Using modified and unmodified triphosphates with unaltered base-pairing specificity and comparing the relative fidelities of incorporation, it is hoped that insights can be obtained. This approach is based on the hypothesis that the difference in catalysis rate of polymerization for good-fit and poor-fit substrates is the basis of the polymerization step discrimination (under normal circumstances poor-fit at the catalytic site is the result of inability to make a good base-pair between template and substrate). Finally, since an understanding of the mutation process is one part of the overall goal of this Institute, learning how DNA polymerases achieve their remarkable accuracy will certainly be an important step in that direction.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60085-01 LEM
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Mechanism of DNA Replication in Procaryotes and Eukaryotes		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: A. Sugino Visiting Associate LEM NIEHS		
COOPERATING UNITS (if any) Department of Biochemistry, University of Chicago, Chicago, Illinois		
LAB/BRANCH Laboratory of Environmental Mutagenesis		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 1	PROFESSIONAL: 1	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) My research interest is to understand the mechanism of DNA replication, especially initiation and elongation of DNA chains. To approach this, I have been using various specific inhibitors of DNA replication, such as nalidixic acid (or oxolinic acid) and coumermycin (or novobiocin). To understand the mechanism of action of these inhibitors on DNA replication, I have tried to isolate and characterize the target proteins.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Purification of target proteins for the inhibitors using in vitro ϕ X174 RF DNA replication complementation assay; agarose gel electrophoresis of relaxed and supertwisted DNA to assay DNA supercoiling activity.

MAJOR FINDINGS AND PROPOSED COURSE: First, I have purified the target protein for nalidixic acid from E. coli using in vitro ϕ X174 RF DNA replication system in collaboration with Dr. N. R. Cozzarelli. We have shown that this protein is a subunit of the DNA supercoiling enzyme (DNA gyrase) which is implicated in DNA replication, transcription, and site-specific recombination. Also, we have recently purified another subunit of DNA gyrase, the target protein for coumermycin or novobiocin, and succeeded in reconstituting all of the enzymatic activities of DNA gyrase. We are now studying the mechanism of DNA supercoiling by DNA gyrase using ATP analogs.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies help to understand the complexity of DNA replication, particularly the importance of the tertiary structure of DNA, and will provide a corresponding understanding of mutagenic mechanisms.

PUBLICATIONS

Higgins, N.P., Peebles, C.L., Sugino, A., and Cozzarelli, N.R.: Purification of the subunits of Escherichia coli DNA gyrase and reconstitution of enzymatic activity. Proc. Natl. Acad. Sci. 75, 1773-1777, 1978.

Sugino, A., Higgins, N.P., Brown, P.O., Peebles, C.L., and Cozzarelli, N.R.: Energy coupling in DNA gyrase and the mechanism of action of novobiocin. Proc. Natl. Acad. Sci. 75, 1978. (in press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60086-01 LEM										
PERIOD COVERED October 1, 1977 to September 30, 1978												
TITLE OF PROJECT (80 characters or less) Biochemical Studies of Mouse Enzymes, I. IDH												
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">C.-Y. Lee</td> <td style="width: 35%;">Senior Staff Fellow</td> <td style="width: 15%;">LEM</td> <td style="width: 10%;">NIEHS</td> </tr> <tr> <td>Other</td> <td>B. Pegoraro</td> <td>Visiting Fellow</td> <td>LEM</td> <td>NIEHS</td> </tr> </table>			PI:	C.-Y. Lee	Senior Staff Fellow	LEM	NIEHS	Other	B. Pegoraro	Visiting Fellow	LEM	NIEHS
PI:	C.-Y. Lee	Senior Staff Fellow	LEM	NIEHS								
Other	B. Pegoraro	Visiting Fellow	LEM	NIEHS								
COOPERATING UNITS (if any) None												
LAB/BRANCH Laboratory of Environmental Mutagenesis												
SECTION												
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709												
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">TOTAL MANYEARS:</td> <td style="width: 33%;">PROFESSIONAL:</td> <td style="width: 33%;">OTHER:</td> </tr> <tr> <td style="text-align: center;">0.4</td> <td style="text-align: center;">0.4</td> <td style="text-align: center;">0</td> </tr> </table>			TOTAL MANYEARS:	PROFESSIONAL:	OTHER:	0.4	0.4	0				
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:										
0.4	0.4	0										
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS												
SUMMARY OF WORK (200 words or less - underline keywords) Two isozymes of NADP⁺-dependent <u>isocitrate dehydrogenase (IDH)</u> are preferentially localized in cytoplasm and <u>mitochondrial</u>, respectively, of various tissues. Genetic variants of the cytoplasmic isozyme have been identified in DBA/2J and in C57BL/6J mice. The purpose of this study is: (1) to provide a simple purification procedure for the isolation of these two isozymes; (2) to compare their structural and biochemical properties, in order to correlate their differential distribution in the tissues or their subcellular localization; (3) to compare biochemically the two cytoplasmic IDH variants from DBA/2J and C57BL/6J mice.												

PROJECT DESCRIPTION

METHODS EMPLOYED: Purification of IDH isozymes by affinity chromatography on 8-(6-aminohexyl)-amino-NADP⁺-Sephacrose and 8-(6-aminohexyl)-amino-AMP-Sephacrose.

MAJOR FINDINGS AND PROPOSED COURSE: The two isozymes were purified to homogeneity and were determined to be dimers with the same molecular weight and sedimentation coefficient. However, they have distinct differences in amino acid composition. They were shown to have similar K_m 's for coenzyme and substrates, pH dependence of activity and sensitivities to inactivation by urea. However, they differ distinctly in coenzyme specificity, thermal stability and sensitivity to alkylating reagents. The two cytoplasmic IDH variants revealed very similar biochemical properties, and only slightly different pH-dependent thermal inactivation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A new and simple procedure has been developed for the purification of IDH isozymes. The biochemical characterization of these two isozymes is an attempt to understand the function of isozymes in the regulation of cell metabolism processes. Comparative studies of the two genetic variants will lead to a better understanding of natural variants for the biochemical screening of mutant enzymes in mice.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60087-01 LEM										
PERIOD COVERED October 1, 1977 to September 30, 1978												
TITLE OF PROJECT (80 characters or less) Biochemical Studies of Mouse Enzymes, II. PGK-B Variants												
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT												
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">C.-Y. Lee</td> <td style="width: 35%;">Senior Staff Fellow</td> <td style="width: 10%;">LEM</td> <td style="width: 5%;">NIEHS</td> </tr> <tr> <td>Other:</td> <td>B. Pegoraro</td> <td>Visiting Fellow</td> <td>LEM</td> <td>NIEHS</td> </tr> </table>			PI:	C.-Y. Lee	Senior Staff Fellow	LEM	NIEHS	Other:	B. Pegoraro	Visiting Fellow	LEM	NIEHS
PI:	C.-Y. Lee	Senior Staff Fellow	LEM	NIEHS								
Other:	B. Pegoraro	Visiting Fellow	LEM	NIEHS								
COOPERATING UNITS (if any) None												
LAB/BRANCH Laboratory of Environmental Mutagenesis												
SECTION												
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709												
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:										
0.5	0.5	0										
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER												
☐ (a1) MINORS ☐ (a2) INTERVIEWS												
SUMMARY OF WORK (200 words or less - underline keywords) Three <u>genetic variants</u> of <u>3-phosphoglycerate kinase-B</u> (a sperm-specific isozyme) were purified respectively from DBA/2J, C3H/HeJ and C57BL/6J mice, and their kinetic properties were compared. <u>Stability</u> of the enzymes was compared at high temperatures, extreme pH's or in the presence of urea and iodoacetate.												

PROJECT DESCRIPTION

METHODS EMPLOYED: Purification of the sperm-specific isozyme from three different strains of mice by affinity chromatography on 8-(6-aminohexyl)-amino-ATP-Sepharose, according to the method developed previously in this laboratory.

MAJOR FINDINGS AND PROPOSED COURSE: Three PGK-B variants examined were found different in two major aspects: the PGK-B variants from DBA/2J and C3H/HeJ mice have very similar kinetic properties, but differ in stability at high temperatures. However, the variant from C57L/J mice is thermally stable but has a low specific activity. In order to clarify the nature of the variation, it is proposed to employ immunological techniques to determine if the low activity variant is the result of a structural or a regulatory mutation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A knowledge of mouse biochemical genetic variants from a biochemical point of view is crucial for mutagenesis testing in mammals.

PUBLICATIONS

Pegoraro, B., and Lee, C.-Y.: Purification and characterization of 2 isozymes of 344 glycerate kinase. Biochem. 522: 423-433, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60088-01 LEM
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Biochemical Studies of Mouse Enzymes, III. Immunology of PGK		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Other:	C.-Y. Lee B. Pegoraro A. Ansari R. Ericson	Senior Staff Fellow Visiting Fellow Visiting Associate LEM NIEHS LEM NIEHS LBG NIEHS
COOPERATING UNITS (if any) Laboratory of Biochemical Genetics, NIEHS Department of Human Genetics, University of Michigan, Ann Arbor, Michigan		
LAB/BRANCH Laboratory of Environmental Mutagenesis		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.6	OTHER: 0
CHECK APPROPRIATE BOX(ES)		
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER		
☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Rabbit antisera were raised respectively against two 3-phosphoglycerate kinase (PGK) <u>isozymes</u> from DBA/2J mice. The purpose of this study is (1) the characterization of the immunological properties of the two PGK isozymes and (2) the comparative immunological study of genetic variants of PGK-B.		

PROJECT DESCRIPTION

METHODS EMPLOYED: By routine immunological and enzyme inactivation techniques.

MAJOR FINDINGS AND PROPOSED COURSE: The rabbit antisera have been tested for specificities and cross-reactivities toward the other isozyme. The antisera will be used for the immunological characterization of genetic variants of PGK-B.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Antisera against PGK-B, a sperm-specific enzyme, could be employed for the study of spermatogenesis and for the labeling of sperms for human mutation-monitoring.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60089-01 LEM															
PERIOD COVERED October 1, 1977 to September 30, 1978																	
TITLE OF PROJECT (80 characters or less) Biochemical Studies of Mouse Enzymes, IV. Isoelectric Focusing																	
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																	
<table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">PI:</td> <td style="width: 30%;">J. Lopez-Barea</td> <td style="width: 30%;">Visiting Associate</td> <td style="width: 10%;">LEM</td> <td style="width: 10%;">NIEHS</td> </tr> <tr> <td>Other:</td> <td>C.-Y. Lee</td> <td>Senior Staff Fellow</td> <td>LEM</td> <td>NIEHS</td> </tr> <tr> <td></td> <td>C. Shore</td> <td>Biological Laboratory Technician</td> <td>LEM</td> <td>NIEHS</td> </tr> </table>			PI:	J. Lopez-Barea	Visiting Associate	LEM	NIEHS	Other:	C.-Y. Lee	Senior Staff Fellow	LEM	NIEHS		C. Shore	Biological Laboratory Technician	LEM	NIEHS
PI:	J. Lopez-Barea	Visiting Associate	LEM	NIEHS													
Other:	C.-Y. Lee	Senior Staff Fellow	LEM	NIEHS													
	C. Shore	Biological Laboratory Technician	LEM	NIEHS													
COOPERATING UNITS (if any) None																	
LAB/BRANCH Laboratory of Environmental Mutagenesis																	
SECTION 																	
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																	
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">TOTAL MANYEARS:</td> <td style="width: 33%;">PROFESSIONAL:</td> <td style="width: 33%;">OTHER:</td> </tr> <tr> <td style="text-align: center;">1.2</td> <td style="text-align: center;">0.3</td> <td style="text-align: center;">0.9</td> </tr> </table>			TOTAL MANYEARS:	PROFESSIONAL:	OTHER:	1.2	0.3	0.9									
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:															
1.2	0.3	0.9															
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																	
SUMMARY OF WORK (200 words or less - underline keywords) <u>Isoelectric points</u> of about 16 mouse enzymes from various tissues were determined by preparative isoelectric focusing column. Strain variations of isoelectric points of some mouse enzymes were detected. We attempted to use isoelectric point determinations as a means to detect new <u>genetic variants</u> in mice that were not identified previously by electrophoresis.																	

PROJECT DESCRIPTION

METHODS EMPLOYED: LKB preparative isoelectric focusing columns were used.

MAJOR FINDINGS AND PROPOSED COURSE: We were able to identify the known genetic variants in DBA/2J and C57BL/6J by their differential isoelectric points. We will search for new genetic variants from liver and kidney tissues from different inbred strains of mice by improving the accuracy of the isoelectric point determinations.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In view of the high resolution of isoelectric focusing measurements, one can accurately determine the isoelectric points of many proteins from mouse tissues. This can be a quantitative means of identifying genetic variants or mutant enzymes in mice.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60090-01 LEM										
PERIOD COVERED October 1, 1977 to September 30, 1978												
TITLE OF PROJECT (80 characters or less) Biochemical Studies of Mouse Enzymes, V. Glutathione Reductase												
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0"> <tr> <td>PI:</td> <td>J. Lopez-Barea</td> <td>Visiting Associate</td> <td>LEM</td> <td>NIEHS</td> </tr> <tr> <td>Other:</td> <td>C.-Y. Lee</td> <td>Senior Staff Fellow</td> <td>LEM</td> <td>NIEHS</td> </tr> </table>			PI:	J. Lopez-Barea	Visiting Associate	LEM	NIEHS	Other:	C.-Y. Lee	Senior Staff Fellow	LEM	NIEHS
PI:	J. Lopez-Barea	Visiting Associate	LEM	NIEHS								
Other:	C.-Y. Lee	Senior Staff Fellow	LEM	NIEHS								
COOPERATING UNITS (if any) None												
LAB/BRANCH Laboratory of Environmental Mutagenesis												
SECTION												
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709												
TOTAL MANYEARS: 0.7	PROFESSIONAL: 0.7	OTHER: 0										
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS												
SUMMARY OF WORK (200 words or less - underline keywords) Glutathione reductase from livers of DBA/2J mice was purified to homogeneity by affinity chromatography. It is a dimeric enzyme of molecular weight 107,000 daltons with a sedimentation coefficient 6.13S, stoke radius 41.8°A and PI = 6.91. Kinetic studies revealed a ping-pong mechanism. The <u>substrate</u> protects the enzyme against <u>thermal inactivation</u> .												

PROJECT DESCRIPTION

METHODS EMPLOYED: N^6 -2¹,5¹-ADP and C8-NADP⁺- Sepharose columns were employed for enzyme purifications by affinity chromatography.

MAJOR FINDINGS AND PROPOSED COURSE: NADPH at low concentrations was found to inactivate the enzyme oxidized glutathione. We also plan to purify a variant of this enzyme from SWR mice. Comparative biochemical studies will be performed between two genetic variants of glutathione reductase to see if one can identify the genetic variations by biochemical assays.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: NADPH inactivation in vivo could be an important regulatory mechanism for detoxification. Comparative study of enzyme variants is important for mutagenesis testing in mice.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER 201 ES 60091-01 LEM										
PERIOD COVERED October 1, 1977 to September 30, 1978												
TITLE OF PROJECT (80 characters or less) Biochemical Studies of Mouse Enzymes and Genetic Variants, VI. Phosphoglucose Isomerase (PGI).												
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">D. Charles</td> <td style="width: 30%;">Visiting Fellow</td> <td style="width: 10%;">LEM</td> <td style="width: 10%;">NIEHS</td> </tr> <tr> <td>Other:</td> <td>C.-Y. Lee</td> <td>Senior Staff Fellow</td> <td>LEM</td> <td>NIEHS</td> </tr> </table>			PI:	D. Charles	Visiting Fellow	LEM	NIEHS	Other:	C.-Y. Lee	Senior Staff Fellow	LEM	NIEHS
PI:	D. Charles	Visiting Fellow	LEM	NIEHS								
Other:	C.-Y. Lee	Senior Staff Fellow	LEM	NIEHS								
COOPERATING UNITS (if any) None												
LAB/BRANCH Laboratory of Environmental Mutagenesis												
SECTION												
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709												
TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.9	OTHER: 0										
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td>☐ (a) HUMAN SUBJECTS</td> <td>☐ (b) HUMAN TISSUES</td> <td>☒ (c) NEITHER</td> </tr> <tr> <td colspan="3">☐ (a1) MINORS ☐ (a2) INTERVIEWS</td> </tr> </table>			☐ (a) HUMAN SUBJECTS	☐ (b) HUMAN TISSUES	☒ (c) NEITHER	☐ (a1) MINORS ☐ (a2) INTERVIEWS						
☐ (a) HUMAN SUBJECTS	☐ (b) HUMAN TISSUES	☒ (c) NEITHER										
☐ (a1) MINORS ☐ (a2) INTERVIEWS												
SUMMARY OF WORK (200 words or less - underline keywords) Genetic variants of <u>phosphoglucose isomerase</u> were purified respectively from muscle extract of DBA/2J and C57BL/6J mice. Structural and kinetic properties of these two electrophoretic variants were compared under different experimental conditions in an attempt to find any other biochemical differences.												

PROJECT DESCRIPTION

METHODS EMPLOYED: A C8-ATP-Sepharose column was employed as a major step of enzyme purification by "affinity elation."

MAJOR FINDINGS AND PROPOSED COURSE: These two electrophoretic variants were found to have a thermal stability difference at 50°. The half-life for the DBA/2J and C57BL/6J enzymes were shown to be 3 min and 1 min, respectively. However, they have identical kinetic parameters and structural characteristics.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This is part of our overall program on the development of methodology for biochemical screening of enzyme variants or mutants in mice.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60092-01 LEM															
PERIOD COVERED October 1, 1977 to September 30, 1978																	
TITLE OF PROJECT (80 characters or less) Biochemical Mutations in Drosophila Induced by Chronic Irradiation																	
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">C. Langley</td> <td style="width: 30%;">Research Geneticist</td> <td style="width: 10%;">LEM</td> <td style="width: 10%;">NIEHS</td> </tr> <tr> <td>Other:</td> <td>R. Voelker</td> <td>Senior Staff Fellow</td> <td>LEM</td> <td>NIEHS</td> </tr> <tr> <td></td> <td>R. Racine</td> <td>Visiting Fellow</td> <td>LEM</td> <td>NEIHS</td> </tr> </table>			PI:	C. Langley	Research Geneticist	LEM	NIEHS	Other:	R. Voelker	Senior Staff Fellow	LEM	NIEHS		R. Racine	Visiting Fellow	LEM	NEIHS
PI:	C. Langley	Research Geneticist	LEM	NIEHS													
Other:	R. Voelker	Senior Staff Fellow	LEM	NIEHS													
	R. Racine	Visiting Fellow	LEM	NEIHS													
COOPERATING UNITS (if any) None																	
LAB/BRANCH Laboratory of Environmental Mutagenesis																	
SECTION																	
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																	
TOTAL MANYEARS: 1.2	PROFESSIONAL: 1.0	OTHER: 0.2															
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																	
SUMMARY OF WORK (200 words or less - underline keywords) A strain of <u>Drosophila melanogaster</u> (SM1/+2) was replicated into 1000 lines and exposed to <u>chronic low-dose-rate γ-radiation</u> (7 rads/hours) for 14 generations (a cumulative dose of 33,552 rads). After scoring electrophoretically for mutants at the 7 soluble enzyme loci <u>Got-2</u> , <u>αGpdh</u> , <u>c-Mdh</u> , <u>Adh</u> , <u>Dip-A</u> , <u>Hex-C</u> and <u>αAmy</u> , 13 independent mutants were found: 5 at the <u>αGpdh</u> loci, 4 at <u>Hex-C</u> , 2 at <u>c-Mdh</u> , 1 at <u>Got-2</u> and 1 at <u>Adh</u> . Analysis of the salivary gland chromosomes showed that of these 13 mutants, only 2 (<u>αGpdh</u>) are associated with <u>chromosome aberrations</u> . This shows that at chronic low-dose-rate irradiation the proportion of cytogenetic aberrations is decreasing. An experiment to study the recessive lethal rate induced under chronic low-dose-rate α -radiation (7 rads/hours, 14 days, cumulative dose of 2352 rads) showed an induced mutation rate of 3.7% for the X-chromosome and 11.7% for the second chromosome. This indicates that chronic low-dose-rate α -radiation leads to a similar number of mutants/rad as acute irradiation.																	

PROJECT DESCRIPTION

METHODS EMPLOYED: A strain of Drosophila melanogaster composed of balanced lethal heterozygotes, SM1/+ λ , was replicated into 1000 lines and brother-sister mated each generation while being exposed to low-dose-rate γ -radiation (7 rads/hour) for 14 generations (a cumulative dose of 33552 rads). This strain is heteroallelic for the soluble enzyme loci Got-2, α Gpdh, c-Mdh, Adh, Dip-A, Hex-C and α -Amy. When electrophoresed after the irradiation, the unmutated flies give codominant phenotypes. If one of the alleles in a particular line has sustained a null mutation (loss of enzyme activity) the phenotype will appear dominant (or homogygous).

MAJOR FINDINGS AND PROPOSED COURSE: 13 mutations were found at different loci: 5 α Gpdh, 4 Hex-C, 2 c-Mdh, 1 Got-2 and 1 Adh. For the Adh and α -Gpdh loci (one each), mutants were found which could be due to gene conversion, a double cross over or a mutation from the original to a form identical in mobility to that found on the other chromosome. The rest of the mutants are apparent nulls. Two other mutants (α -Gpdh, c-Mdh) produce gene products and are thus point mutations. Both have altered mobilities and loss of mutant homodimer activity. Three mutants, (α -Gpdh, c-Mdh and Got-2), produce no functional hetero- or homodimers as detected from gel staining.

The mutant salivary gland chromosomes were also analyzed. Of the 13 mutants, 11 show no indication of being associated with chromosome aberrations. Two α -Gpdh mutants show an altered chromosome pattern. One of them shows a deficiency, the other an inversion in the α -Gpdh region. Of special interest is that the 2 α -Gpdh mutants associated with chromosomal aberrations are hemizygous lethal.

This experiment suggests that at chronic low-dose-rate irradiation, the proportion of cytogenetic deletions is low. In order to get an estimate of the mutation rate induced by chronic low-dose-rate γ -radiation, a recessive-lethal experiment was carried out for the X and 2nd chromosomes. One generation (from egg to adult fly) was chronically irradiated at a low dose rate (7 rads/hour) for 14 days (a cumulative dose of 2352 rads). Induced recessive lethal rates for the X and 2nd chromosomes of 3.7% and 11.7%, respectively, are found. This recessive lethal experiment indicates that chronic γ -radiation leads to a similar number of mutants per rad as acute irradiation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60093-01 LEM								
PERIOD COVERED October 1, 1977 to September 30, 1978										
TITLE OF PROJECT (80 characters or less) Selection for Recombination Rates in <i>Drosophila melanogaster</i>										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 20%;">PI:</td> <td style="width: 30%;">B. Charlesworth</td> <td style="width: 30%;">Visiting Scientist</td> <td style="width: 20%;">LEM NIEHS</td> </tr> <tr> <td>Other:</td> <td>D. Charlesworth</td> <td>Visiting Scientist</td> <td>LEM NIEHS</td> </tr> </table>			PI:	B. Charlesworth	Visiting Scientist	LEM NIEHS	Other:	D. Charlesworth	Visiting Scientist	LEM NIEHS
PI:	B. Charlesworth	Visiting Scientist	LEM NIEHS							
Other:	D. Charlesworth	Visiting Scientist	LEM NIEHS							
COOPERATING UNITS (if any) None										
LAB/BRANCH Laboratory of Environmental Mutagenesis										
SECTION 										
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709										
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:								
2.0	2.0	0								
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (a1) MINORS ☐ (a2) INTERVIEWS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER										
SUMMARY OF WORK (200 words or less - underline keywords) Selection for increased and decreased recombination is being practiced in two intervals of the third chromosome of <i>Drosophila melanogaster</i> . If selection is successful, genetic analyses of the factors involved will be carried out. This should increase our understanding of the nature of the genetic control of one of the most fundamental of biological processes - <u>genetic recombination</u> .										

PROJECT DESCRIPTION

METHODS EMPLOYED: Selection for both increased and decreased recombination is being practiced, using two intervals on chromosome 3 of *Drosophila melanogaster*. The intervals are defined by three dominant genetic markers.

The technique is to select progeny each generation that represent recombination or nonrecombination events depending on the direction of selection. This should lead to selection for genes on the other chromosomes that increase (or decrease) the level of recombination between the three markers. The selected genetic variation in recombination will then be analyzed by standard *Drosophila* mapping techniques.

MAJOR FINDINGS AND PROPOSED COURSE: At present, enough generations have not been completed to draw any conclusions. It is planned that after 10 generations the individual selection lines will be examined to determine the extent and genetic nature of any changes in recombination. An important finding will be the generality (or specificity) of the factors that are isolated. Some may increase (or decrease) recombination throughout the genome while others may affect only the selected region on the third chromosome.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: An understanding of the nature of genes affecting recombination that are found in natural populations will help in understanding, in evolutionary terms, how recombination rates may be controlled. This fundamental genetic process is one that is the subject of renewed attention by both theoretical and experimental geneticists.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60094-01 LEM
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Tissue Specificity of Sister Chromatid Exchange (SCE) Formation		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: J. Allen Senior Staff Fellow LEM NIEHS		
COOPERATING UNITS (if any) J. A. McLachlan, Genetics Division, Children's Hospital Medical Center and Harvard Medical School		
LAB/BRANCH Laboratory of Environmental Mutagenesis		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 1.2	PROFESSIONAL: 0.7	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline) The <u>in vivo</u> 5-bromodeoxyuridine (BrdU)-dye method is particularly useful for cytogenetic analyses of <u>tissue-specific</u> levels of <u>chromosome mutagenesis</u> . Differences in baseline and mutagen-induced frequencies of <u>sister chromatid</u> <u>exchange</u> (SCE) for somatic and germ cells have been demonstrated. In the present study, <u>in vivo</u> approaches will be modified in order to extend SCE analyses to additional tissues. Further clarification of germ cell responsiveness and maturation following mutagen exposure will also be attempted.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Various in vivo approaches will be used to incorporate and detect BrdU in diverse replicating tissues. These methods will provide continuous exposure of analogue to animals through series of BrdU injections or through BrdU depot schemes. In some instances, radioactive labelling will be done in order to monitor cell progression.

MAJOR FINDINGS AND PROPOSED COURSE: Tissues will be compared as to the extent of their SCE responsiveness to different mutagens; and the nature of discrepancies will be investigated in this project and in subsequent projects. Germ cell maturation will also be studied following BrdU substitution and mutagen insult.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In vivo analyses of SCE permit assessment of genetic damage following host metabolism, and thus possible activation or deactivation of the test compound. The high relevance of this test system should be further enhanced by a more thorough understanding of differential tissue sensitivities. The behavior of critical cell types, such as developing germ cells, following exposure to SCE inducing agents is of particular importance in this regard.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60095-01 LEM
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Sister Chromatid Exchange Studies in Rodent Pre- and Post-Implantation Embryonic Tissues		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Other	J. W. Allen W. Sheridan H. Michelmann M. Sanyal E. El-Nahass	Senior Staff Fellow Mammalian Geneticist Visiting Fellow Reproductive Physiologist Visiting Fellow
		LEM NIEHS LEM NIEHS LEM NIEHS LEM NIEHS
COOPERATING UNITS (if any) Laboratory of Environmental Toxicology, NIEHS		
LAB/BRANCH Laboratory of Environmental Mutagenesis		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 1.4	PROFESSIONAL: 0.9	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Sister chromatid exchange (SCE) analyses, using 5-bromodeoxyuridine (BrdU) dye techniques in adult mammalian tissues, have provided highly sensitive indices of chromosome mutagenesis by mutagens and/or carcinogens. In the present work, we propose to adapt BrdU techniques for cytogenetic studies of SCE formation, and also replication kinetics in pre- and post-implantation mouse and/or rat embryonic tissues. Studies will be conducted with selected teratogens.		

PROJECT DESCRIPTION

METHODS EMPLOYED: A variety of in vitro and in vivo cytological methods will be employed in an effort to grow embryonic cells in the presence of BrdU. Attainment of sufficient analogue incorporation to effect sister chromatid differentiation should permit baseline SCE estimates and allow analysis of SCE increments and replication patterns following exposure to a test compound.

MAJOR FINDINGS AND PROPOSED COURSE: Following establishment of appropriate conditions for studying SCE in embryo tissues, mutagen/teratogen responsiveness will be studied under direct (culture) exposure conditions, and under host-mediation (exposure of intact parent, pre- or post-fertilization) conditions. A variety of physiological factors influencing the transmission and formation of SCE during developmental stages will be considered and may prove of special interest.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The high sensitivity of SCE analysis makes it an important test system for detecting genetic damage resulting from environmental chemicals. The present study should provide insights into the basic sensitivity of embryos to this form of DNA alteration, whether induced or inherited, following exposure to teratogenic substances.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60096-01 LEM										
PERIOD COVERED October 1, 1977 to September 30, 1978												
TITLE OF PROJECT (80 characters or less) Comparison of Rates of Induction of Point Mutations and Chromosome Aberrations in Cultured Cells												
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI:</td> <td style="width: 33%;">W. Sheridan</td> <td style="width: 33%;">Research Geneticist</td> <td style="width: 10%;">LEM</td> <td style="width: 10%;">NIEHS</td> </tr> <tr> <td>Other:</td> <td>R. Sorg</td> <td>Geneticist</td> <td>LEM</td> <td>NIEHS</td> </tr> </table>			PI:	W. Sheridan	Research Geneticist	LEM	NIEHS	Other:	R. Sorg	Geneticist	LEM	NIEHS
PI:	W. Sheridan	Research Geneticist	LEM	NIEHS								
Other:	R. Sorg	Geneticist	LEM	NIEHS								
COOPERATING UNITS (if any) D. Clive, Burroughs Wellcome, Research Triangle Park, North Carolina												
LAB/BRANCH Laboratory of Environmental Mutagenesis												
SECTION												
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709												
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:										
0.7	0.2	0.5										
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS												
SUMMARY OF WORK (200 words or less - underline keywords) In order to assess the utility of various test systems, different aspects of their potential should be explored. If one type of <u>genetic damage</u> is more easily detected within a system, this might be the criterion of choice. Using the Mouse <u>Lymphoma cell line L5178Y</u> , mutations at the <u>TK locus</u> were induced by chemical treatment. Studies utilizing the same <u>mutagens</u> are conducted on the frequencies of induction of <u>chromosomal damage</u> and aberrations, and compared with the <u>point mutation</u> frequency.												

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard methods of culture of L5178Y Mouse Lymphoma cells are employed. The chemical treatments are applied in the standard manner. Cytogenetic preparations are made by standard techniques.

MAJOR FINDINGS AND PROPOSED COURSE: Analysis to date indicate that there is a correlation between the induction of point mutations and the induction of chromosomal aberrations. Whether this correlation is coincidental or whether there is a real relationship is currently being considered. Planned studies include cytogenetic analysis of specific mutant cell lines recovered in the point-mutation studies.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The need for efficient biological detectors of genetic effects from environmental mutagens can be filled only when the detection system has been validated. Such validation can to some extent be arrived at by using several different genetic end points, and comparing their efficiencies, so that information regarding the basic mechanisms and relationships underlying induction of genetic damage may be deduced.

NEW YORK MEDICAL COLLEGE - Valhalla, New York
(NIH-NIEHS-75-29)

TITLE: Tier One Microsomal Assay for Mutagenesis

CONTRACTOR'S PROJECT DIRECTOR: Herbert S. Rosenkranz, Ph.D.

PROJECT OFFICER (NIEHS): George R. Hoffmann, Ph.D., Geneticist, LEM
Errol Zeiger, Ph.D., Research Microbiologist, LEM

DATE CONTRACT INITIATED: March 11, 1975

CURRENT ANNUAL LEVEL: \$186,252

PROJECT DESCRIPTION

OBJECTIVES: The purposes of this project are: (1) the testing of chemicals for mutagenicity in bacteria both with and without a mouse liver microsomal activation system; (2) development of a computer control system for quality control in bacterial suspension tests and for data analysis; (3) evaluation of the computerized testing format under actual experimental conditions.

METHODS EMPLOYED: Highly trained technicians will follow computerized protocols in the testing of chemicals for mutagenicity in a blind study. The data analysis will be done via a computerized system and the feasibility of more extensive use of such a computerized system will be evaluated.

MAJOR FINDINGS AND PROPOSED COURSE: Testing chemicals under this contract is in progress. Since this is a blind study, however, data analysis has not yet been done. Work done during the past 9 months has indicated that certain modifications are required in the computer control system and laboratory protocols. This developmental work is now in progress.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The applicability of computerized systems in mutation testing will be determined. Simultaneously, data will be obtained relevant to mutagenicity test system validation and the mutagenicity of compounds of both theoretical and applied interest.

UNIVERSITY OF BRITISH COLUMBIA - Vancouver, British Columbia
(NIH-75-C-638)

TITLE: Testing Compounds for Mutagenic Activity in a Meiotic
Non-disjunction Test in Neurospora crassa

CONTRACTORS PROJECT DIRECTOR: A. J. F. Griffiths, Ph.D.

PROJECT OFFICER (NIEHS): F. J. de Serres, Ph.D.
Associate Director for Genetics

DATE CONTRACT INITIATED: June 16, 1975

CURRENT ANNUAL LEVEL: \$16,500

PROJECT DESCRIPTION

OBJECTIVES: The objective of this contract is to assay environmental chemicals for mutagenic activity in a meiotic non-disjunction test in Neurospora crassa.

METHODS EMPLOYED: The contractor has developed two strains of Neurospora crassa with a series of genetic markers in each on Linkage Group I (LGI). When these two strains are crossed to each other and ascospores are plated (about 10^6 ascospores per cross) on minimal medium, the only ones that can grow are disomic for LGI. The aneuploid condition is unstable and breaks down to form a heterokaryon composed of two (haploid) nuclear types representing the original disomic pair of chromosomes. This heterokaryon is called a pseudowild-type and segregates vegetatively into the two components; thus, pseudowild-type frequency is an index of the frequency of meiotic non-disjunction involving LGI in this organism.

MAJOR FINDINGS AND PROPOSED COURSE: Control platings (untreated crosses) have shown that spontaneous non-disjunction frequencies are quite constant with an average value of 4.9×10^{-5} . Treatment is made by adding test chemicals to the crossing medium. All stages of meiosis will be affected. After plating ascospores from control and treated crosses, a comparison is made of pseudowild-type frequencies. A total of 10 chemicals have been found which give significantly higher frequencies of non-disjunction than found in control crosses: p-fluorophenylalanine, trifluralin, trimethoprin, caffeine, atrazine, resorcinol, stilphosterol, 2,4-diaminoanisole S, alloxan, 8-hydroxyquinoline. In addition, gamma rays were also found to give a positive test.

Experience with this assay system has shown that it can provide a useful assay for non-disjunction. Statistical analysis of the data indicates that at least five tests have to be performed on each compound in order to obtain statistically reliable data. A single technician under the supervision of the Project Director can perform about 125 tests per year or, with 5 tests per compound, can test 25 chemicals per year.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

In order to develop a truly comprehensive battery of tests for mass screening programs, we must be able to detect all classes of genetic alterations. No such assay exists for non-disjunction. Because of this we only have scanty evidence of chemicals which will cause non-disjunction. The assays with Neurospora have detected activity in 10 chemicals out of 62 tested. This is an important class of genetic alteration with regard to genetic disease in man.

Because of the marked specificity of some chemical mutagens, it is considered essential to continue work with the Neurospora assay to evaluate its utility and feasibility as an assay system in mass screening programs.

PUBLICATION

Griffiths, A. J. F., and Delange, A. M.: p-Fluorophenylalanine Increases Meiotic Non-Disjunction in a Neurospora Test System. Mutation Research 46: 345-354, 1977.

MILES LABORATORY - Elkhart, Indiana
(NIH-N01-ES-3-2113)

TITLE: Genetic Tests to Characterize Specific Locus Mutants in Neurospora crassa

CONTRACTOR'S PROJECT DIRECTOR: J. L. Colbourn, Ph.D.

PROJECT OFFICER (NIEHS): Tong-man Ong, Ph.D., Geneticist, LEM

DATE CONTRACT INITIATED: April 9, 1973

CURRENT ANNUAL LEVEL: \$69,963

PROJECT DESCRIPTION

OBJECTIVES: The primary objective of this contract is the genetic characterization of presumptive adenine-3 (ad-3) mutants of Neurospora crassa derived from forward-mutation experiments at the Institute and at Illinois State University (under contract with the Institute) with various environmental chemicals as well as various chemical carcinogens.

METHODS EMPLOYED: Stock cultures of presumptive ad-3 mutants are characterized by a series of heterokaryon tests with various standard tester sets which determine the genotypes of the mutants, the complementation pattern of ad-3B mutants showing allelic complementation, and also distinguish point mutations from chromosome deletions.

MAJOR FINDINGS AND PROPOSED COURSE: During the past year more than 2500 mutants were made homokaryotic for adenine requirement, and silica gel stock cultures were prepared. Mutants which have been successfully characterized include those induced by ICR-170 in haploid wild-type and in six different haploid UV-sensitive strains and those induced by various chemical carcinogens, and various two-component heterokaryons. Mutants which will be analyzed in the future include (1) carcinogen-induced mutants in excision-repair-deficient strains, (2) carcinogen-induced mutants in standard wild-type strains, and (3) carcinogen-induced mutants in the two-component heterokaryon homozygous for excision repair-deficient strains, as well as in heterokaryon 12. This contract will be terminated in April, 1978.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This contract relates to various programs in this Institute where it is essential to characterize the mutants induced at specific loci in this organism with regard to the type of lesion (e.g., point mutation or chromosomal deletion as well as the nature of the damage to the DNA at the molecular level). This is essential in our program to determine the correlation between carcinogenic and mutagenic activity as well as to determine whether chemical carcinogens characteristically produce particular types of lesions at the molecular level. In addition, the characterization of the specific-locus mutants produced by environmental chemicals enables us to better determine their relative hazard for man.

THE JACKSON LABORATORY - Bar Harbor, Maine
(NIH-N01-ES-4-2156)

TITLE: Development of a System for Detecting Lethal Mutations in Mice

CONTRACTOR'S PROJECT DIRECTOR: Thomas H. Roderick, Ph.D.

PROJECT OFFICER (NIEHS): W. Sheridan, Ph.D., Mammalian Geneticist, LEM

DATE CONTRACT INITIATED: January 15, 1974

CURRENT ANNUAL LEVEL: \$75,000

PROJECT DESCRIPTION

OBJECTIVES: At present no efficient test system exists for detection of point mutations in mammals. The aim of the contract is to develop strains of mice, carrying marked inversions, which may be used in studies of induction of point mutations. Theoretically these tests will be comparable to the system presently used in *Drosophila*. The prime goal is to induce inversions in as many chromosomes of the mouse as possible and combine them in a number of strains to be used as tester stocks.

METHODS EMPLOYED: The contractor induces chromosome breaks and inversions by means of radiation and/or chemical agents. He mates the treated animals and in the F_1 does cytological analysis of one testis of each individual male. If a significant increase in anaphase bridges appears, this is taken as an indication that the animal may carry an inversion; and he is mated to get offsprings for further study. Following cytological and linkage studies to characterize the aberration, a decision is made as to the utility of the inversion and appropriate marker genes are introduced into the stock.

MAJOR FINDINGS AND PROPOSED COURSE: The contractor has induced and recovered 29 inversions. Of these, 19 have been thoroughly characterized for linkage and chromosomal morphology. One of the inversions has been successfully utilized in tests to detect induced recessive lethal mutations. The estimated length of the contract is five years. Efforts will continue to induce and characterize further inversions. It is proposed to combine inversions with Robertsonian Translocations to increase the effectiveness of the system.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Recessive lethal mutations are known to exist at high frequencies in human populations. Many environmental compounds are known to be mutagenic and capable of causing both chromosome aberrations and point mutations. With an efficient animal model to study the induction of such mutations, more accurate estimates can be made of the risks such compounds imply to the human population.

BROOKHAVEN NATIONAL LABORATORY - Upton, New York
(Interagency Agreement)

TITLE: Chemical and Environmental Mutagen Studies Utilizing the
Tradescantia Test System

CONTRACTOR'S PROJECT DIRECTOR: Jack Van't Hof, Ph.D.

PROJECT OFFICER (NIEHS): F. J. de Serres, Ph.D.
Associate Director for Genetics

DATE CONTRACT INITIATED: June 6, 1972

CURRENT ANNUAL LEVEL: \$350,000

PROJECT DESCRIPTION

OBJECTIVES: The primary objective of this program is to determine the feasibility of using the Tradescantia stamen hair system to detect the mutagenicity of gaseous and particular air pollutants. Tradescantia was chosen because of the breadth of radiobiological data on the somatic mutation system, the versatility and relative ease of handling experimental material, high sensitivity to both chemical and physical mutagens and its small number (12) of large chromosomes suitable for cytological analysis. Moreover it can be exposed directly to ambient air pollutants.

METHODS EMPLOYED: To exploit the Tradescantia system in pursuit of the above objectives, work has progressed in three main areas: definition of the basic genetics of Tradescantia, elucidation of the mechanisms responsible for mutation-induction and field exposures to ambient air pollution.

Genetic and plant breeding studies are continuing in an effort to clarify the genetic basis of the flower color marker and to develop new clones with improved characteristics.

The Tradescantia system is being utilized in applied studies (testing of new compounds) in both laboratory and field exposures although the major effort during this December to June period has been on field studies. New chemicals are being screened individually for mutagenicity under laboratory conditions and facilities are being prepared to explore the possibility of synergistic reactions between mutagens. Applied studies have been extended into the field through the use of a Mobile Monitoring Vehicle (MMV) which has been fabricated and is undergoing field testing in high pollution areas throughout the country. Pollution site selection and field exposures are being carried out in collaboration with EPA.

MAJOR FINDINGS AND PROPOSED COURSE: Genetic and plant breeding studies are continuing in an effort to clarify the genetic basis of flower color changes in the stamen hair and petal cells of *Tradescantia* and to develop new clones with improved characteristics.

The genetic studies include several projects related to the genetics on the inheritance of white, pink and blue flower color as well as the genetic analysis of the particular alleles used in the heterozygous clones 02 and 4430.

The plant breeding studies are designed to evaluate the performance of a newly selected dwarf heterozygote in the Mobile Monitoring Vehicle. In addition new loci are being incorporated into standard clones to extend the test system to include both somatic and genetic mutations for screening. A particularly important project in this area is the establishment of new clones better adapted for experimental mutagenesis than the present clones 02 and 4430.

Major emphasis during the past year has been placed on field trials using the Mobile Monitoring Vehicle. Sites visited in the period December 16, 1976 to June 15, 1978 include Elizabeth, NJ, Charleston, WV, Birmingham, AL and Baton Rouge, LA. The results of the four trials indicate that the *Tradescantia* stamen hair system has shown a positive response to ambient air in Elizabeth, Charleston and Baton Rouge but not in Birmingham. Mutagenic response may be correlated loosely with ambient pollution from petroleum and chemical processing but not steel smelting. No other correlations can be made until data from other sites and validated pollution measurements (to be provided by the EPA Mobile Monitoring Unit) are available.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

The *Tradescantia* stamen hair system is the most sensitive assay system which has been developed to detect the mutagenic activity of environmental chemicals with regard to the production of gene mutations. The simplicity of both the exposure procedure and the scoring of stamen hairs to determine mutation frequency rates is especially well suited for both basic studies as well as field applications for monitoring air pollutants in general or highly specific areas (i.e. monitoring air pollutants in industries producing biologically active chemicals). The sensitivity of this assay system makes it especially well suited for monitoring air pollutants as they actually occur and avoids the complexities associated with collecting condensates and evaluation of eluted samples from the condensates. Evaluation of ambient air pollutants is essential to evaluate possible synergistic effects between chemicals which may or may not be biologically active per se.

PUBLICATIONS

McNulty, P. J., Nauman, C. H., Sparrow, A. H., Schwemmer, S. S. and Schairer, L. A.: Influence of x-ray dose fractionation on the frequency of somatic mutations induced in *Tradescantia* stamen hairs. Mutation Research. 44: 235-246, 1977.

Ichikawa, S., Sparrow, A. H., Nauman, C. H. and Takahashi, C. S.: Influence of radiation dose rate on somatic mutation frequency and loss of reproductive integrity in *Tradescantia* stamen hairs. Mutation Research, in press.

Nauman, C. H., Sparrow, A. H. and Schairer, L. A.: Uptake of tritiated 1,2-dibromoethane by *Tradescantia* floral tissues: relation to induced mutation frequency in stamen hairs. Mutation Research, in press.

Nauman, C. H. and Scott, B. R. : Mutagenicity of some brominated aliphatic compounds. Mutation Research, Rev. Genet. Toxicology, in press.

Sparrow, A. and Schairer, L. A.: The effects of chemical mutagens (EMS & DBE) and specific air pollutants (O_3 , SO_2 , NO_2 , N_2O) on somatic mutation rates in *Tradescantia*.

Geneticheskie Posledsviya Zagrayazneniya Okruzhayushchei Sredy (Genetic Effects of Pollution in the Environment), Akad. Nauk. SSSR - Moscow, 1977

Nauman, C. H., Schairer, L. A. and Sparrow, A. H.: Influence on temperature on spontaneous and radiation-induced somatic mutations in *Tradescantia* stamen hairs. Mutation Research. 50: 207-218, 1978.

Ma, Te-Shiu, Sparrow, A. H., Schairer, L. A., Nauman, Anne S.: Effects of 1,2-dibromoethane in DBE on Meiotic Chromosome of *Tradescantia*. Mutation Research, in press.

Scott, B., Sparrow, A. H., Schwemmer, S. C., Schairer, L. A.: Plant Metabolic Activation of 1,2-dibromoethane (EDB) to a Mutagen Potency Mutation Research. 49: 203-212, 1978.

LABORATORY OF ENVIRONMENTAL TOXICOLOGY

LABORATORY OF ENVIRONMENTAL TOXICOLOGY Summary Statement

The scientific efforts of the Laboratory of Environmental Toxicology (LET) are directed toward the prevention of environmentally-related diseases through understanding mechanisms of toxicity. Special emphasis is placed upon environmental factors which may adversely affect reproduction and development. Toxicologic phenomena are studied from the descriptive to the molecular level in search of a better understanding of mechanisms. This increased understanding contributes to the development of better and more effective test procedures to predict toxicity in humans. Available scientific expertise is broad and includes biochemistry, reproductive physiology, teratology, pharmacology, cell biology, pathology, and behavior. Test systems are likewise diverse and range from molecular biochemistry and electron microscopy, through *in vitro* cell, organ, and embryo cultures, to whole animals. Whether their study be molecular and/or biochemical mechanisms of toxic action, pharmacology and pharmacokinetics in developing systems, the identification of sensitive biochemical indicators which predict specific organ damage, or some other area of toxicology, scientists in LET are in a unique position to develop and utilize basic scientific information for resolving critical environmental health problems.

The research goals of the Laboratory are supported by both intramural scientists and contracted research. The scope of the overall effort is broad involving both national and international programs and adjunct appointments. Recognizing training as one of its primary responsibilities, the Laboratory supports graduate students at nearby universities, offers training opportunities for foreign postdoctoral scientists and American scientists on sabbatical leave. In short, the Laboratory of Environmental Toxicology integrates research and training into a program that is both worldwide in scope and one of the strongest multidisciplinary efforts in toxicology in this country.

EXPERIMENTAL TERATOGENESIS

The research of this group seeks a better understanding of teratogenic compounds, particularly their molecular mechanisms of action. Scientists are currently testing the hypothesis that certain drugs and environmental chemicals interfere with embryogenesis by irreversibly combining with critical molecules in the fetus. As a first step to investigate this possibility, known acylating agents of simple chemical structure, including anhydrides and imides, are being used as test compounds. To determine the teratogenicity and embryotoxicity of these agents, each is administered to pregnant CD-1 mice. One

day prior to full term, fetuses are removed and examined for gross, visceral or skeletal abnormalities. All of the acylating agents thus far tested have produced malformations in the offspring of treated dams.

In order to correlate teratogenic potential with chemical reactivity, the interaction of acylating agents with fetal and maternal macromolecules in vivo and proteins and model nucleophiles in vitro is being studied. Following the administration of ^{14}C phthalic anhydride in vivo, covalently bound radioactive material has been found in the tissues of both the mother and the conceptus. In the in vitro culture system of rat embryos during the organogenesis phase of development, rat conceptuses of pregnancy days 10, 11 or 12 have been grown for two days with extensive differentiation of major organs; treatment of these embryos with teratogens have induced developmental anomalies.

TERATOLOGY

This group is concerned with the environmental agents that cause birth defects in mammals. A large part of this group's effort is devoted to the development of an Environmental Teratology Information Center (ETIC), providing the biomedical community access to all publications that contain data pertinent to the teratogenic potential of environmental agents or conditions to which man is exposed. This program will help in defining priorities for future investigations and in preventing unplanned duplication of research. The first block of information is now available on-line as a part of the National Library of Medicine's ToxLine.

Since modern man is exposed to a multitude of physical, chemical and infectious agents, most of which have not been tested for teratogenic potential, this group is also concerned with the study of teratogenic effects of environmental agents. Results of studies on the teratogenicity of benzene, toluene, and o-, p-, and m-xylene in mice are being analyzed. In collaboration with the Laboratory of Environmental Biophysics, pregnant mice exposed to various types of noise (alone and in combination) and to electromagnetic irradiation are presently being studied.

MOLECULAR EMBRYOLOGY

This workgroup is concerned with defining and analyzing certain molecular aspects of the differentiation process in early mammalian development and at later stages of organogenesis for the purpose of identifying sensitive and specific indicators of toxicity. In particular, research concentrates on analyzing patterns of gene expression at the transcriptional and translational level in the model embryo body/teratocarcinoma system (OTT 6050) in the mouse. In this system, the embryo bodies, which consist of an outer ring of endoderm and an inner mass of embryocarcinoma cells, grow as ascites in mice. Frequently, embryo bodies "implant" on the liver, spleen, or mesentery and this stimulates the production of teratocarcinomas, predominantly neuroepithelial in nature.

Our approach isolates total poly(A)-containing RNA from embryoid bodies, teratocarcinomas, and the F9 embryocarcinoma cell line grown in vitro. These RNA preparations are then characterized with respect to poly(A) content, molecular size, and length of the poly(A) tract. Complementary DNA probes are prepared to these RNA preparations and the sequence and abundance of classes of these preparations are being defined. Results indicate that the RNA sequence populations in both embryoid bodies and teratomas can be divided into three classes, low, middle, and high complexity, with the tumors expressing about 1.5 times more information in each RNA sequence class.

By hybridizing the complementary DNA from the teratocarcinomas with embryoid body poly(A)-RNA and vice versa, the degree of sequence overlap can be obtained between the two states of differentiation. Approximately 85% of the poly(A)-RNA sequence set overlap between embryoid bodies and teratocarcinomas. Moreover at least 50% of the abundant sequence class (those poly(A)-RNAs that are in the highest concentration in the cell) are specific for the teratocarcinomas and are not present in the embryoid bodies.

Another area of research involves the utilization of in vitro translation systems for isolating and purifying specific messenger RNAs. Studies using both one-dimensional as well as two-dimensional polyacrylamide gel electrophoresis have detected major changes in alpha-fetoprotein (AFP) from teratocarcinomas. AFP was identified using antibody to AFP isolated and purified from rat amniotic fluid. An antibody to bovine brain specific protein S-100 has been prepared to investigate this protein in neuroepithelial teratocarcinomas. This antibody will also be used to identify S-100 producing cells in in vitro cultures of differentiating embryoid bodies. Likewise the glial fibrillary acidic protein (GFA) from cow has been purified and the production of antibody is underway. This antibody will be used to identify differentiating astrocytes derived from embryocarcinoma cells and in the identification and purification of the messenger RNA for GFA.

TRANSPLACENTAL TOXICOLOGY

This group has demonstrated that prenatal exposure to DES adversely affects the reproductive capacity of the male and female offspring of mice. Such prenatal exposure results in a low incidence of female genital tract neoplasms including vaginal and uterine adenocarcinoma. Similar lesions could not be produced following prenatal exposure to the steroidal estrogen, 17β -estradiol. Differential fetal protein binding between DES and estradiol may help explain these results.

Scanning electron microscopy (SEM) of the genital tract of in utero exposed females has revealed surface ultrastructural abnormalities which may precede neoplastic changes. SEM may provide a tool for early detection of such changes. These studies are being correlated with observations on the role of steroid receptors in reproductive tissue alterations. In studies with male offspring from DES treated mice, a high incidence of testicular and sperm abnormalities was detected. The fate of Mullerian duct remnants in DES treated males is being determined and the role of these tissues in

prostatic lesions evaluated. The distribution, metabolism, and structure-activity relationships of DES in perinatal systems have been determined. The role of toxication-detoxication processes is being evaluated in specific reproductive tract lesions. These studies emphasize the biological significance of potentially activated metabolites. These studies should aid in extrapolating these results to other classes of estrogenic environmental chemicals.

DEVELOPMENTAL TOXICOLOGY

This group is concerned with perinatal aspects of enzymology, endocrinology, and pharmacology. A multidisciplinary effort is utilized in attempts to identify sensitive biochemical indicators of developmental toxicity having clinical value and to understand the mechanisms responsible for alterations in normal enzyme development. Emphasis is placed on the hormonal regulation of enzyme development in control and treated animals and the role of altered hormone action in developing systems. Biochemical and morphological changes are correlated with the uptake, binding, metabolism, accumulation, and clearance of foreign compounds and hormones in whole animals and culture systems. Current interests center on liver, intestine, reproductive tract, and the pituitary-hypothalamic axis.

Structure-activity relationships have been defined for the perinatal pharmacology of pure PCB and PBB congeners including effects on hepatic enzyme development. 3,4-3',3'-Tetrachlorobiphenyl (4-CB) induces P-448 dependent enzymes in 6 and 20 day-old rats whereas 2,4,5-2',4',5'-hexachlorobiphenyl (6-CB) induces P-450 dependent enzymes. 4-CB is a potent teratogen (behavioral and kidney teratology) but 6-CB produces no such developmental effects. 4-CB might also alter the normal imprinting of sexual differentiation of hepatic metabolism as evidenced by a feminization (decrease) of UDPGT in adult male rats exposed neonatally to 4-CB. Therefore, the organohalogenes might alter the ontogeny of hepatic metabolism in two ways: induction and imprinting.

Research efforts are in progress to assess the liver as a target organ for estrogens including the characterization of hepatic cytosolic and nuclear estrogen receptors and to elucidate the mechanisms of perinatal imprinting of hepatic metabolism by hormones and hormonally-active xenobiotics such as the PCBs. Treatment of newborn rats with DES or TP produces no change in the prepubertal activities of several hepatic enzymes (5 α -reductase, 16 α -hydroxylase, histidase, UDP glucuronyltransferase, ethylmorphine demethylase, etc.). However, the subsequent adult animals exhibit masculinized or feminized levels of enzyme activity and these effects appear to be permanent and irreversible. Imprinting of enzyme activity is apparently mediated through the pituitary-hypothalamic axis during a critical period of early development. Further experiments show that sensitivity to the toxic effects (liver and testes) of cadmium are permanently altered qualitatively and quantitatively by newborn exposure to DES.

Another area of research focuses on hormonal and environmental factors affecting intestinal function and energy-producing processes during perinatal development and seeks to identify biochemical components indicative of normal

and/or altered intestinal function. One study has shown that intestinal pyruvate dehydrogenase (PDH) is converted to its active form immediately after birth allowing for increased utilization of pyruvate by the TCA cycle resulting in increased ATP production necessary for active transport to occur in the developing intestine. PDH activity and the activation process are highly sensitive to oral arsenic exposures. β -Glucuronidase isozyme profiles in rats exposed to dimethylhydrazine, a specific colon carcinogen, may provide an early enzyme indicator for dimethylhydrazine-induced intestinal carcinogenesis. LDH isozymes are also being investigated as indicators of intestinal toxicity. An intestinal primary culture system formed by the isolation of tip and crypt cells is being developed and evaluated as a possible *in vitro* model for cellular differentiation, intestinal toxicity, and intestinal detoxication-detoxication of environmental chemicals.

DEVELOPMENTAL PATHOLOGY

This program seeks to determine the postnatal toxic effects on offspring of rats exposed to chemicals throughout gestation. Offspring are evaluated at various stages of development from newborn to geriatric. In addition to gross observations, the disposition of the chemical as well as effects on organ system function, immunological competence, learning and behavior are determined. Gross, histological, and subcellular pathology in offspring of treated and control females is analyzed. The offspring's adaptive ability and endurance capacity are also determined.

Current studies demonstrate that methyl-n-butyl ketone (MBK) and metabolites are present in both the fetal and maternal compartments in term pregnant rats. MBK-exposed females exhibited a 12 to 24 hour delay in parturition. Offspring of exposed animals, although similar at birth, show a progressive delay in development at all stages tested during the first six months. Biochemical changes and the mechanisms responsible need to be identified.

Females treated with enflurane delivered a larger percentage of small litters than did controls; however, birth weight and subsequent growth rate of offspring were comparable to controls. Clinical chemistry tests in weanling pups revealed an increased creatinine phosphokinase activity and elevated triglyceride level, but these increases were not evident at puberty or adult animals. With regard to ethchlorvynol, weanling animals of treated females had an elevated blood glucose and a decrease in phosphorus, creatinine phosphokinase, SGPT, SGPT and in $\text{NA} - [\text{CL} \pm \text{CO}_2]$ balance. In puberty pups blood glucose and triglycerides were elevated whereas creatinine phosphatase and sodium balance were decreased.

The characterization of potential organ pathology in offspring exposed *in utero* and the delineation of the total spectrum of postnatal toxicity as well as the mechanisms responsible for these effects are challenging areas of study in environmental health science.

GERM CELL DEVELOPMENT

Group scientists are investigating the effects of environmental chemicals on germ cell toxicity. New approaches to reproductive toxicity testing are being explored and validated while other investigations are directed towards the elucidation of mechanisms of toxicity. Rat testes are used to study (1) DNA repair synthesis and DNA breaks as an indicator for chemical mutagens; (2) toxication-detoxication reactions of polycyclic hydrocarbons in gonadal tissue; and (3) induction of MFO enzymes and other marker enzymes in prostate gland by environmental chemicals.

Alkaline elution analysis is a sensitive method for detecting DNA single strand breaks and/or cross-links in mammalian germ cells. Kinetics of alkaline elution of DNA single strand breaks, stabilization of DNA and/or increased DNA repair synthesis in germ cells after chemical exposure have been found to be reliable indicators of toxicity. Recent investigations of testicular microsomal and soluble enzymes demonstrate that both aryl hydrocarbon activating and deactivating enzymes are present. Developmental patterns of these enzymes in rat testes indicate that glutathione S-transferase activity in prepubertal rats is three-four times greater than that of the liver enzyme. In contrast, epoxide hydrazase activities of both liver and testes is very low in prepubertal rats but dramatic increases occur at the onset of puberty. In vitro perfusion of the rat testis with tritium labeled benzo(a)pyrene 4,5-oxide indicated that the active benzo(a)pyrene metabolite is quickly conjugated or converted to a dihydrodiol derivative. The rates of detoxication via these two pathways suggest first order kinetics; the reactions are concentration dependent. Furthermore, in vitro perfusion of testis provides a unique model system for the integration of molecular, biochemical and physiological data with pharmacokinetic parameters. This approach for the study of environmentally-induced alterations of male reproductive organs should serve as an important model for extrapolation to human problems.

Scientists are also attempting to develop a workable fertilization system using human sperm and hamster ova as a model to test for altered fertility induced by environmental agents in humans or laboratory animals. Cleavage to the two-cell stage has been accomplished in a defined medium as well as in a serum supplemented medium, but improvement of the penetration efficiency and/or rates of fertilization is needed.

BIOCHEMICAL TOXICOLOGY

Scientists in this workgroup study basic biochemical and physiological mechanisms through which environmental agents exert their toxic effects in mammalian tissues. The goal is to apply this information to designing rapid and effective test procedures for predicting toxicity in man.

Principal efforts are oriented towards investigation of the mechanisms of regulation of the biosynthesis and physiological disposition of heme and heme-containing proteins in mammalian tissues. Particular emphasis is placed

on understanding the mechanisms by which environmentally important chemicals interact with these processes to effect toxicity in human populations. Specific studies include investigations of differences in regulatory and biochemical properties of various heme biosynthetic pathway enzymes in mammalian tissues, and the role of heme and various metals in the regulation of those enzyme systems. Particular attention has been given to understanding the physical and regulatory properties of σ -aminolevulinic acid (ALA) synthetase, the rate-limiting enzyme in heme biosynthesis, in adult and fetal tissues. Investigation of the pathophysiological circumstances under which other heme biosynthetic pathway enzymes can become rate-limiting in this process during prolonged exposure to hematotoxic environmental agents has also been undertaken. Recent efforts have been oriented toward the development of assay procedures for uroporphyrinogen decarboxylase and coproporphyrinogen oxidase, two heme biosynthetic pathway enzymes which have not as yet been measured, and toward understanding the specific effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on heme biosynthesis and mixed function oxidase activity in mammalian tissues.

Other scientists are attempting to define the early ultrastructural and biochemical effects of heavy metals and other environmental agents on mammalian tissues to derive early warning tests for predicting sub-toxic human responses to such agents. Their studies include determining the correlation between subcellular morphologic observations and biochemical alterations in tissues of animals chronically exposed to low levels of toxic agents for various periods. In current studies, researchers are examining the toxicity of arsenic, cadmium, lead, methyl and inorganic mercury, dioxin (TCDD), and vinyl chloride. Results of their experiments indicate that specific morphological and biochemical response profiles can be established which differentiate various metals and other agents in our multi-element environment. Studies are currently in progress to use these profiles to develop test procedures that will permit identification of preclinical exposure to specific environmental substances in human populations.

COLLABORATIVE RESEARCH

NIH CONTRACTS

Laboratory scientists are Principal Investigators in a wide variety of research contract efforts. These collaborative efforts are logical extensions of the Laboratory's intramural programs concerned with reproductive, developmental, and target organ toxicities.

Dr. Staples has contracted with DOW Chemical Company and the Research Triangle Institute to determine the teratogenic potential of environmental agents in animals. Test agents are applied singly or in combination to more than one species in an effort to discover teratogenic effects as well as possible synergistic actions and/or susceptible populations. Experiments are designed to increase the resolving power of tests which detect potential teratogenic hazards to humans.

Drs. Lucier and McLachlan are collaborating with the Wisconsin Alumni Research Foundation to search for the transplacental toxicity of selected environmental chemicals. This contract emphasizes reproductive tract function and transplacental carcinogenesis and fulfills a critical need to identify chemicals or classes of chemicals that might adversely affect reproductive tract function following gestational exposures. These chemicals can then be studied in detail in the intramural program, focusing on the mechanisms of toxic action. Chemicals currently under study include zeranol, several PCB and PBB isomers, DDE, polycyclic hydrocarbons, and a series of mycotoxins.

Dr. Fowler supervises a contract with the University of Washington to investigate the potential carcinogenic actions of petroleum hydrocarbons using selected estuarine organisms as the model. This research is exploring the relationship between tumor induction and metabolism of the chemical carcinogen, benzo(a)pyrene. The exposure system has been developed, the microsomal enzyme assay system optimized, and the tumor biopsy procedure refined.

Dr. Woods is extending the Laboratory's Target Organ Toxicity efforts through a research contract with Midwest Research Institute. Animals exposed chronically to vinyl chloride (VC) and vinylidene chloride (VD) are evaluated and general toxicity and carcinogenic potential defined. Data derived from these studies are being utilized in the design of models for risk assessment in humans.

The Developmental Toxicology workgroup (Dr. Lucier) and Brigham Young University are attempting to identify clinically-useful biochemical indicators of developmental toxicity. The approach is to first investigate the ontogeny of key enzyme systems following treatment with known teratogens.

COLLABORATIVE RESEARCH WITH ACADEMIC COMMUNITY AND GOVERNMENTAL AGENCIES

There are numerous examples of collaborative research projects at the local, national and international level. Laboratory scientists have acknowledged the resources and expertise concentrated in nearby universities. Moreover, we have not hesitated to establish the collaborative efforts necessary to develop quality intramural programs with scientists throughout the world, including government, academia, and private industry.

Dr. Woods and the NIEHS Biometry Branch are collaborating with the Department of Epidemiology, UNC School of Public Health, on the utilization of animal toxicity data in the design of models for carcinogen risk assessment in human populations exposed to vinyl chloride.

Dr. Fowler and NOAA scientists are investigating the physiological effects of arsenic, cadmium, and copper on marine shellfish. This effort is directed at elucidating the mechanisms by which commercially-important marine shellfish concentrate heavy metals. An unusual cadmium-binding protein has been isolated from shellfish, an increased incidence of copper-containing lysosomes

noted in copper-treated shellfish tissues, and the toxic actions of cadmium and copper on gill respiration are being examined.

Dr. Lucier is studying the role of hormone receptors in xenobiotic-induced alterations of hormone responsiveness with Dr. Shihadeh Nayfeh of the UNC Department of Biochemistry. This project should yield information important to understanding the mechanism of action of important forms of developmental toxicity. In a related project, Drs. Lucier, Lamartiniere, and Fiegelson (Roosevelt Hospital, New York) are attempting to utilize hepatic histidase as a marker indicative of perinatal imprinting of hepatic metabolism. Dr. Schiller is working with Peter Ingram (RTI) on morphological analysis of intestinal cells by SEM.

Dr. McLachlan has several collaborative projects involved with a detailed exploration of the transplacental toxicity of DES and other hormonally-active chemicals. He and Dr. Lamb of the UNC Cancer Research Center are utilizing SEM as a tool to detect early preneoplastic lesions in animals exposed to DES during gestation. Research projects with Dr. Gerald Cunha of the University of Colorado Medical Center, and Dr. Manfred Metzler of the University of Wurzburg, West Germany, are attempting to identify pharmacological properties and cellular alterations important to unravelling the mechanism of DES-induced transplacental carcinogenesis. Additional studies involving Drs. McLachlan, Korach, and Metzler are assessing the role of metabolism in DES toxicity.

Dr. Lee is studying the toxic actions of trace metals and their effects on plasma gonadotropins and androgens. In an international collaboration, Dr. Lee and scientists from the Institute of Toxicology and the University of Zurich are developing a new method for accurately identifying mutagens and carcinogens through an in vivo animal model.

Dr. Dixon has continued a productive collaboration with Dr. Sergio Fabro of the Columbia Hospital for Women. Dr. Fabro has been active in the Experimental Teratogenesis workgroup which is concerned with mechanisms of teratogenesis.

Drs. Dixon, Staples, Lee, and McLachlan are also involved in the US-USSR collaborative research programs in environmental health concerning transplacental toxicity and chronic organ toxicity.

Drs. Harris and Silverberg are collaborating with Dr. Conn of the Duke Pharmacology Department in an effort to purify messenger RNA for LH from sheep pituitary.

NATIONAL AND INTERNATIONAL PROGRAMS:
SYMPOSIA ORGANIZED/COMMITTEE APPOINTMENTS, ETC.

Another important indicator of peer approval and scientific relevance of current Laboratory programs is the frequency that LET scientists organize and participate in "state of the art" symposia and are asked to serve on various

committees attempting to provide meaningful direction in environmental health research. Descriptions of representative examples of Laboratory involvement in this regard are given below.

Dr. Dixon has a large number of committee and program assignments which augment and are relevant to the NIEHS mission such as the Pharmacology-Toxicology Research Committee of the NIGMS, Chairman of the Committee on Environmental Pharmacology of ASPET, Committee on Women in the Workplace organized by NIOSH, Project Director of the teratogenic and reproductive effects areas of the NIEHS Energy-Related Research Program, US-USSR Problem II Coordinator of the Joint Committee for Health Cooperation-Environmental Health involving chronic organ toxicity, Council of the Pan-American Medical Association Section on Environmental Health Sciences, and the Toxicology Review Panel of WHO's Expanded Programme of Research Development and Research Training in Human Reproduction. For the past year, Dr. Dixon has been on a part-time detail to the Office of Science and Technology Policy (OSTP) in the Executive Office of the President in Washington. This position involves Dr. Dixon in a variety of federal activities involving the regulation of toxic substances such as the Interagency Regulatory Liaison Group (IRLG), the Council of Environmental Quality's Toxic Substances Coordinating Committee (TSCC), the development of a federal Cancer Policy, while also assisting the President's Science Advisor with a variety of environmental and toxicologic problems. In addition, Dr. Dixon has been co-organizer of several Target Organ Toxicity Symposia in cooperation with the Society of Toxicology. Proceedings of these symposia are published in Environmental Health Perspectives and include symposia on liver, kidney, lung, gonads, development, nervous system, and cardiovascular system. Dr. Dixon is also a Councilor for the Society of Toxicology.

Dr. Schiller is the NIEHS representative to the NIH Digestive Diseases Coordinating Committee as well as Nutrition Coordinating Committee. She is also chairman of the committee on In Vitro Research and Human Values of the Tissue Culture Association and recently organized a symposium on the Scientists' Responsibilities in the Acquisition and Utilization of Human Tissue for In Vitro Research. In addition, Dr. Schiller is Federal Women's Program coordinator at NIEHS.

Dr. Woods is a representative to DHEW's Toxicology Information Subcommittee, which coordinates toxicology and related programs in DHEW, and serves with the EPA's Hazardous Waste Management Division, Office of Solid Waste, in the implementation of the Resource Conservation and Recovery Act of 1976. Dr. Woods also serves as NIEHS representative to the International Conference on Hepatotoxicity Due to Drugs and Chemicals, as a member of the research panel of CEQ's Federal Working Group on Pest Management, and is a member of the program committee of the Society of Toxicology.

Dr. Fowler has been active in national and international programs dealing with metal toxicity. He was a rapporteur at a recent conference held at the Karolinska Institute entitled "Air Pollution and Cancer: Risk Assessment Methodology and Epidemiology Evidence" and was a co-organizer of International

Conferences on Arsenic and Cadmium Toxicity. Dr. Fowler is serving on the ERDA "Health Effects Panel on Trace Contaminants from Coal Combustion Systems."

Dr. McLachlan is a member of the DHEW Task Force on DES toxicity and has been advisor to NIOSH and the FDA on the toxicity of estrogens.

Dr. Staples' work involving communication of teratological information has led to interactions with ERDA, NLM, NBS, and NICHD. He is Secretary and Councilor-elect for the Teratology Society and was recently named as liaison scientist between the Teratology Society and the Society of Toxicology. Dr. Staples is the U. S. representative for Teratology in the US-USSR Collaboration on Environmental Health.

Dr. Lucier is a member of the Toxicology Forum Panel, which is making recommendations regarding protocol development for in utero toxicity testing. He has been an invited participant and session chairman at numerous conferences and workshops involving developmental pharmacology and toxicology. Dr. Lucier also served as Chairman of the Search Committees for the Chiefs of the Biophysics and Pharmacokinetics Laboratories of the NIEHS and was a member of the Search Committee for the Scientific Director of NIEHS.

INFORMATION EXCHANGE

Communication of basic and applied information vital to environmental health problems is aided by establishing mechanisms for information exchange and by assuming editorial responsibilities. In this regard, Dr. Staples has established the much-needed Environmental Teratology Information Center (ETIC) in collaboration with ERDA and Oak Ridge. Information on teratology is collected, categorized and made available to the biomedical community via TOXLINE. Dr. Staples is also on the Editorial Boards of Environmental Health Perspectives and the Journal of Environmental Health Sciences.

Dr. Lucier is the editor of Environmental Health Perspectives. Dr. Dixon is on the Editorial Boards of Environmental Health Perspectives, Toxicology and Applied Pharmacology, Journal of Pharmacology and Experimental Therapeutics, and the Journal of Environmental Sciences and Health. In addition, LET scientists are frequently asked to review manuscripts for journals oriented toward Biochemistry, Pharmacology, Toxicology, and Teratology.

TRAINING PROGRAMS

Environmental health is a new and demanding research area that is undergoing rapid change and growth. Consequently, there is a growing need for training to ensure adequate numbers of qualified and dedicated researchers in environmental health research. The Laboratory of Environmental Toxicology recognizes this need and our scientists are encouraged to participate in a wide variety of training programs, including adjunct appointments at nearby universities, supervision of graduate student research, development of graduate courses in environmental health, and participation in the Fogarty Visiting Program.

Several LET scientists have adjunct appointments with nearby universities. Dr. Lucier is Adjunct Associate Professor in the Biochemistry Department of the UNC School of Medicine; Dr. Staples is Adjunct Associate Professor of Pharmacology in the Pharmacology Department of the UNC School of Medicine; Dr. Fowler is Adjunct Assistant Professor in the Pathology Department of UNC School of Medicine; Dr. Schiller is Adjunct Assistant Professor of Biochemistry in the UNC School of Medicine and is also on the faculty of the W. Alton Jones Cell Science Center, Lake Placid, New York.

Drs. Schiller and Lucier are involved in organizing a graduate course in Biochemical Toxicology to be taught by the UNC Biochemistry Department.

Laboratory scientists have also been active in the training of graduate students. Graduate students are working in the laboratories of Drs. McLachlan, Lucier, and Dixon. Drs. Schiller and Lucier are supervising the graduate research of Winifred Curley (UNC Biochemistry Department). Postdoctoral training is strongly supported by LET, and our commitment to these programs involves the support of twelve Visiting Fellows and three Visiting Associates.

Drs. Lucier, McLachlan, Schiller, Korach, and Harris have lectured at UNC and/or Duke in areas of their research expertise.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70001-02 LET
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) The Teratogenicity of Antiepileptic Drugs and Chemically Related Compounds*		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	R. L. Dixon	Laboratory Chief
OTHER:	N. A. Brown	Visiting Fellow
	J. Kao	Visiting Fellow
		LET NIEHS
		LET NIEHS
		LET NIEHS
COOPERATING UNITS (if any) Columbia Hospital, George Washington University		
LAB/BRANCH Laboratory of Environmental Toxicology		
SECTION Experimental Teratogenesis Group		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	2	2
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Recent clinical observations have suggested that certain <u>antiepileptic drugs</u> may exert significant <u>teratogenic effects</u> in man. We have evaluated the teratogenic potential of several such drugs using the CD-1 mouse as the animal model. The antiepileptic drugs tested fall into three classes, being derivatives of the simple, cyclic imides; <u>hydantoin</u> , <u>succinimide</u> and <u>oxazolidine-2,4-dione</u> . Following intraperitoneal administration on gestational days 8, 9 and 10 or 11, 12 and 13, all the drugs and the three base compounds were embryotoxic and produced a range of congenital malformations. Notable among the abnormalities recorded were a variety of great vessel defects produced by many of the compounds with particularly high incidences being induced by Trimethadione. There was good agreement between the types of malformations produced in mice with those reported following human exposure indicating the CD-1 mouse is a good model to study teratogenic actions of this class of compounds. In addition, the common teratogenic potential and the structural similarities between these chemicals suggest a common receptor or a mutual mechanism of action.		
*Formerly, The Teratogenicity of Acylating Agents		

PROJECT DESCRIPTION

METHODS EMPLOYED: The test compounds were administered to pregnant CD-1 mice on days 8, 9 and 10 or 11, 12 and 13 of gestation. The chemicals were injected i.p. or given by gavage as a suspension in 0.5% methyl cellulose, control animals receiving vehicle only. Dams were sacrificed on day 18 (day 1 being determined by the presence of copulatory plugs) and offspring were delivered by Cesarean section. The number and uterine positions of all resorbed, dead and live fetuses were recorded and live young were examined for gross malformations. Fetal heads were fixed in Bouins solution for subsequent examination by Wilson's free hand slicing technique. Using a soft tissue technique, described by Staples, the viscera of each fetus was examined and the sex noted. Fetuses were then cleared and stained by the KOH-Alizarin Red S method for skeletal examination.

MAJOR FINDINGS AND PROPOSED COURSE: The antiepileptic drugs tested are chemically related to simple, cyclic imides. Ethotoin, Mesantoin and Diphenylhydantoin are derivatives of hydantoin; Phensuximide, Methsuximide and Ethosuximide are succinimides; and Trimethadione and Paramethadione are of the oxazolidine-2,4-dione class of compounds. All of the drugs and each of the base compounds exerted teratogenic and embryotoxic effects. Following administration on gestational days 11, 12 and 13, cleft palate was the most common abnormality produced while treatment on days 8, 9 and 10 caused primarily skeletal and cardiovascular defects. A range of great vessel malformations was produced by several of the drugs and each of the simple imides; Trimethadione treatment gave a particularly high incidence. Based upon their teratogenic potential, the three classes of drugs may be ranked in the following order; oxazolidine diones>hydantoins>succinamides.

SIGNIFICANCE TO BIOCHEMICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Anticonvulsant therapy has been suggested to be connected with increased incidences of human birth defects. However, such conclusions are largely based upon retrospective analyses of records, and the etiology of abnormalities is often confused by the influence of epilepsy *per se*. This study shows that anticonvulsant drugs are teratogenic in the CD-1 mouse, producing a spectrum of malformations which includes many of the abnormalities recorded following human exposure. Thus, the CD-1 mouse may be a good animal model for use in studies of the teratogenicity of anticonvulsants. Since these drugs and their base compounds all exert similar embryotoxic and teratogenic effects and since they all possess the imide structure, this suggests that there may be a common receptor for these compounds or at least a common mechanism of action.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70010-02 LET
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Study of Developmental Disorders in a Model of Embryo Culture During Organogenesis*		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: OTHER:	M. K. Sanyal N. A. Brown E. El-Nahass R. L. Dixon G. O. Emerole	Reproductive Physiologist Visiting Fellow Visiting Fellow Laboratory Chief Visiting Associate
		LET NIEHS LET NIEHS LET NIEHS LET NIEHS LET NIEHS
COOPERATING UNITS (if any)		
Columbia Hospital for Women, Washington, D. C.		
LAB/BRANCH Laboratory of Environmental Toxicology		
SECTION Experimental Teratogenesis Group		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0
CHECK APPROPRIATE BOX(ES)		
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER		
☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
In order to detect and study the mechanisms by which some environmental agents induce developmental disorders, an <u>in vitro culture system</u> of rat <u>embryos</u> during the <u>organogenesis</u> phase of development (<u>paralleling in vivo</u> development) has been developed. In this culture system, rat conceptuses of pregnancy days 10, 11 or 12 have been grown for two days. These embryos grow <u>in vitro</u> 5-20 fold with extensive differentiation of <u>major organs</u>, e.g., brain, neural tube, sensory organs, circulatory system, <u>gut</u>, <u>liver</u>, <u>musculature</u> etc. comparable with those <u>in vivo</u> during the same period. Treatment with <u>teratogens</u>, <u>TEM</u>, <u>BUDR</u>, and <u>hep-arin</u> have been shown to induce different types of <u>developmental anomalies</u> in these embryos grown <u>in vitro</u>.		
(*Formerly, Postimplantation Embryo Culture: A Model for the Study of Organogenesis)		

PROJECT DESCRIPTION

METHODS EMPLOYED: Rat embryos are being cultured during the period of organogenesis when the embryos are most susceptible to agents which may induce developmental anomaly. Conceptuses dissected out of the mother on pregnancy days 10, 11 or 12 are cultured in heat-inactivated homologous plasma, serum or serum fortified with amino acids, glucose and B vitamins for 2-3 days at 38°C in rotating bottles in gas phases appropriate for the stage of development. Teratogens are added to the culture medium to test their effects on organogenesis of the embryo. In vitro development of the embryos is evaluated by morphological (gross, histology and EM), biochemical (DNA, RNA, protein etc.), and cytogenetic (karyotyping, sister chromatid exchange) parameters.

MAJOR FINDINGS AND PROPOSED COURSE: (1) A precise sequence of O₂ requirement with progressive growth of embryos has been developed which provides optimal "normal" development.

(2) Homologous serum extracted from male rats of 75-80 days age, centrifuged immediately, was found to be a superior medium for in vitro embryo growth than heparinized plasma poor in platelets. During the process of clotting, serum is presumably enriched with nutrients and growth promoting factors which are beneficial for the growth and differentiation of the embryos.

(3) Association of components of culture medium, accumulation of excretory materials, depletion of nutrients, partial pressures of gases, and pH with growth and differentiation of embryos has been studied. Several factors detrimental to embryonic growth (e.g., urea accumulation, increased pCO₂, low pO₂, low pH) and nutritional requirements (e.g. glucose, aminoacids) have been recognized.

(4) Effects of teratogens, TEM, BUdR, and heparin on in vitro development and organ differentiation of embryos are being studied currently. Preliminary results indicate that these compounds are capable of inducing different types of developmental disorders, including malformation of somites, sensory organs, neural tubes and brain development.

Our immediate objectives are:

(a) to devise methods for culturing embryos at stages of very early organogenesis with continued differentiation and growth by providing specific nutritional and hormonal requirements.

(b) to study effects of environmental chemicals, potential teratogens and carcinogens on growth and differentiation of embryos in vitro utilizing morphological and biochemical parameters.

(c) to study relationship of chromosomal aberrations and sister chromatid exchange with chemically induced developmental disorders. Methods for these studies are currently perfected for evaluating the effects of model teratogens on the embryos developed in vitro.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A significant percent of developmental anomalies in humans is presumably due to exposure to adverse environmental factors. It is also known that most major organ malformations appear during the critical stage of organogenesis. An embryo culture system which parallels normal development during the period of organogenesis under controlled conditions provides opportunities to study cellular and molecular processes associated with normal and abnormal development. Studies on effects of teratogens and carcinogens in this in vitro model will be useful in prediction of potential environmental agents which may induce birth defects, and mechanisms by which teratogens induce developmental disorders.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70020-06 LET
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Effect of Environmental Agents on Ontogenesis After Parental Exposure		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: OTHER:	R. E. Staples P. S. Nawrot R. O. Cook D. I. McRee	Research Physiologist Visiting Fellow Acoustical Engineer Research Physicist
		LET NIEHS LET NIEHS LEB NIEHS LEB NIEHS
COOPERATING UNITS (if any) Research Triangle Institute Department of Nutrition, PHS, University of North Carolina at Chapel Hill		
LAB/BRANCH Laboratory of Environmental Toxicology		
SECTION Teratology Group		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 3.5	PROFESSIONAL: 1.2	OTHER: 2.3
CHECK APPROPRIATE BOX(ES)		
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER		
☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The long range purpose of this project is to determine the <u>developmental toxicity</u> and <u>teratogenic potential</u> of selected <u>environmental agents</u> in mammals by application of the test agents to the parent(s) before mating or to the dam during gestation. Work on the chemicals listed previously has been terminated and manuscripts have been published or are being prepared. Studies are being conducted on various types of <u>noise</u> up to 130dB, on <u>microwave</u> , and on <u>solvents</u> . The <u>mouse</u> is used as the experimental animal.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Test agents were given to pregnant females of four species: mouse, rat, hamster, and rabbit by two or more routes, e.g., per os, subcutaneous, intrauterine, during various periods of ontogenesis. Doses were selected on the basis of preliminary study. Those which produced a given pharmacological response (maximum tolerated dose, comparable blood levels, 50% fetal mortality, etc.) were selected. Stresses or disease states that may be elicited before mating were also studied for teratogenic potential either alone or in combination with other environmental components. An attempt was made to compare the sensitivity of various approaches. Qualitative or quantitative differences or similarities in teratogenic response were noted. Resulting offspring were examined for alterations in the incidence of external, visceral, and skeletal changes shortly before term.

MAJOR FINDINGS AND PROPOSED COURSE: Mice were exposed 12 hours daily to 113dB of very high frequency noise (Rodogard®), to which rodents are particularly sensitive. From Days 1 through 6 of gestation, significantly fewer mice maintained pregnancy to term than control mice merely housed in the sound chamber for the same period of gestation. However, maternal weight gain and fetal survival among the remaining female mice were not adversely affected.

Mice exposed to the same noise from Days 6 through 15 of gestation maintained their pregnancies to term as well as the chamber control group; no adverse maternal effects of the noise were observed. However, significantly more fetuses in the exposed group died in later pregnancy than in the chamber control group. The incidence of malformed fetuses was not significantly increased among the groups exposed to noise, and corticosterone levels measured in maternal blood by a sensitive radioimmunoassay procedure did not reveal an association with fetal survival.

Studies on the embryotoxic and teratogenic potential of low power density (5 and 21 mW/cm²) electro magnetic irradiation (2450 MHz) in the mouse have been completed. The incidence of malformations was not increased, but the remaining results have to be analyzed. Additional studies with a higher power level (30 mW/cm²) or electro magnetic irradiation are almost complete.

Results of studies on the teratogenic potential of benzene, toluene, and o, p, and m xylene in the mouse are being analyzed. Protocols to measure peak maternal blood levels and peak fetal levels attained with solvents are being prepared.

A manuscript on the noise study has been drafted for review.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Modern man is exposed continuously to a multitude of chemical and infectious insults as well as to physical stresses. Most of these agents have not been tested

for teratogenic potential. Through the efforts described, the teratogenic potential of selected environmental agents is evaluated in animals. Jet engine and sonalert noise were studied since more than 3500 women of child-bearing age are currently being exposed to this type of noise at up to 130dB in the United States alone. Likewise, exposure associated with microwaves and industrial solvents appear to be increasing, and their potential hazards have not been studied adequately.

PUBLICATIONS

Schneider, U. and Maurer, R. R.: Asbestos and embryonic development. Teratology 15, 3: 273-280, 1977.

Maurer, R. R., Bank, H. and Staples, R. E.: Pre- and postnatal development of mouse embryos after storage for different periods in cryogenic temperatures. Biol. Reprod. 16, 2: 139-146, 1977.

Lu, M. H. and Staples, R. E.: Teratogenicity of ethylenethiourea and thyroid function in the rat. Teratology 17, 2: 171-178, 1978.

Staples, R. E. and Goulding, E. H.: Dipterex teratogenicity in the rat, hamster, and mouse when given by gavage. Envir. Health Perspect. (in press).

Staples, R. E.: Teratogenic screening: Current approaches. Envir. Health Perspec. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70045-02 LET																				
PERIOD COVERED October 1, 1977 to September 30, 1978																						
TITLE OF PROJECT (80 characters or less) Teratocarcinoma System as a Model for Gene Expression in Normal and Abnormal Development*																						
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																						
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 40%;">S. E. Harris</td> <td style="width: 25%;">Senior Staff Fellow</td> <td style="width: 10%;">LET</td> <td style="width: 10%;">NIEHS</td> </tr> <tr> <td>OTHER:</td> <td>A. B. Silverberg</td> <td>Staff Associate</td> <td>LET</td> <td>NIEHS</td> </tr> <tr> <td></td> <td>D. B. Carter</td> <td>Staff Fellow</td> <td>LET</td> <td>NIEHS</td> </tr> <tr> <td></td> <td>P. E. Mansson</td> <td>Visiting Fellow</td> <td>LET</td> <td>NIEHS</td> </tr> </table>			PI:	S. E. Harris	Senior Staff Fellow	LET	NIEHS	OTHER:	A. B. Silverberg	Staff Associate	LET	NIEHS		D. B. Carter	Staff Fellow	LET	NIEHS		P. E. Mansson	Visiting Fellow	LET	NIEHS
PI:	S. E. Harris	Senior Staff Fellow	LET	NIEHS																		
OTHER:	A. B. Silverberg	Staff Associate	LET	NIEHS																		
	D. B. Carter	Staff Fellow	LET	NIEHS																		
	P. E. Mansson	Visiting Fellow	LET	NIEHS																		
COOPERATING UNITS (if any)																						
None																						
LAB/BRANCH Laboratory of Environmental Toxicology																						
SECTION Molecular Embryology Group																						
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																						
TOTAL MANYEARS: 6	PROFESSIONAL: 4	OTHER: 2																				
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																						
SUMMARY OF WORK (200 words or less - underline keywords)																						
The mouse <u>embryoid body/teratocarcinoma</u> system OTT 6050 developed by Leroy Stevens appears to be an ideal system for studying the <u>molecular mechanism</u> of cellular differentiation in a mammalian system. Total <u>poly(A)-RNA</u> was isolated from simple embryoid bodies and teratomas derived from the embryoid bodies. By <u>RNA-complementary DNA hybridization analysis</u>, an evaluation of the <u>sequence complexity</u> of the preparations could be made by <u>computer analysis</u>. <u>Three classes of poly(A)-containing RNA</u> could be resolved in both embryoid bodies and teratomas based on their relative abundance in the respective RNA preparations. By similar analysis <u>nuclear poly(A)-RNA</u> was analyzed. Antibodies to brain specific proteins, S-100 and <u>glial fibrillary acidic protein</u> are being prepared to be used as probes for gene expression during neuroepithelial differentiation in the teratocarcinoma system.																						
(*Formerly, Sequence Complexity of Poly(A)-RNA Isolated From Mouse Embryoid Bodies and Teratomas)																						

PROJECT DESCRIPTION

METHODS EMPLOYED: Embryoid bodies and teratomas were grown, collected and transferred into 129 Sv-S1 mouse peritoneal cavities every two to three weeks. Poly-(A)-containing RNA was prepared by affinity chromatography using oligo-dT cellulose. Poly(A) content was determined by hybridization with excess ^3H -poly(dT) with known standards and determination of amount of hybrid using the S1 nuclease assay. The size of the poly(A)-containing RNA was determined on 70% formamide sucrose gradients by hybridization across the gradient with the ^3H poly(dT). By RNaseA and T-1 treatment of the RNA preparations and native polyacrylamide gel electrophoresis with known standards, the length of the A tract was determined. ^3H -complementary DNA was synthesized using reverse transcriptase, sized and fractionated on alkaline sucrose gradients. Nucleic acid hybridizations were carried out under standard conditions. The hybridization curves were resolved into a number of first order reactions (RNA excess).

Nuclear RNA of embryoid bodies was prepared from citric acid nuclei and the poly(A⁺)-heterogeneous (Hn) RNA was isolated using poly(U)-Sephadex. The above methods for characterizing total poly(A⁺)-RNA (mainly cytoplasmic) were then applied to the nuclear poly(A)-Hn RNA.

Slab gel polyacrylamide electrophoresis has been used to assay in vivo and in vitro protein synthesis patterns, to monitor purification of rat and pig brain specific proteins, and to monitor purification of teratoma specific proteins.

Tissue culture techniques were used to study the growth and differentiation of embryoid bodies in vitro.

Brain specific proteins were purified from pig and rat brains using the 100,000 xg supernatant as starting material. Ammonium sulfate fractionation, size fractionation on Sephadex and ion-exchange separation methods were used in the purification of the proteins. Similar techniques were used in the purification of mouse teratoma specific proteins.

Antibodies to various pure proteins have been raised in rabbits using multiple site injection techniques.

MAJOR FINDINGS AND PROPOSED COURSE: The poly(A⁺)-RNA preparation representing mainly cytoplasmic poly(A⁺) in RNA contains 2.9-5.6% poly(A), a number average size of 1200 to 1400 nucleotides and a poly(A) tract length of 70-80 nucleotides (NT). The ^3H -complementary DNA synthesized was about 350 NT for both preparations, and a cut was taken from the alkaline gradient of material 400-1000 NT. These ^3H -C-DNAs were then used in the hybridization analysis. The embryoid body hybridization curve was resolved by computer into three classes of sequences. The first or most abundant class represented 10% of the total

sequences and from its rate of hybridization is only one or two sequences (of approximate complexity of 1300NT). The second or middle class represents 60% of the total sequences and is some 150 different sequences. Finally, the last or most complex class represents about 25% of the total hybridization and some 4,000 different RNA sequences.

A similar analysis of the teratoma total poly(A)-containing RNA hybridization curve indicated again three major classes of sequences: (1) first class representing 20% and containing two to five different sequences (2) middle class representing 54% and some 200 different sequences and (3) the high complexity class representing some 6,000 different sequences and about 20% of the total sequences expressed.

By hybridization of ^3H -C-DNA from teratomas with poly(A⁺)-RNA from embryoid bodies and the reciprocal experiment, it was established that approximately 85% of the RNA sequences are common or overlap between the undifferentiated embryoid bodies and in the neuroepithelial teratomas. From analysis of these hybridization curves it appears as if approximately half of the most abundant class in the teratomas may be specific to the teratomas and either absent in the embryoid bodies or in a much lower concentration.

Nuclear poly(A⁺)-Hn RNA has been isolated from the embryoid bodies. The number average size is approximately 2,500 NT on formamide sucrose gradients and the poly(A)-tract is about 120-150 NT. We are presently making the ^3H -complementary DNA to begin the hybridization complexity analysis.

Pig brain specific protein S-100 and rat alpha-fetoprotein (AFP) have been isolated and purified. Rabbit antibodies are presently being prepared. The brain specific protein, glial fibrillary acid (GFA) protein, has been partially purified by hydroxy apatite chromatography.

Since the embryoid bodies differentiate into teratomas containing a high concentration of neuroepithelial cells, such as glial, neural, and ependymal, we hope to use the antibodies to S-100, AFP and GFA as probes for expression of these genes during teratocarcinoma differentiation. The antibodies will be used in in vitro translation systems for identification and purification of their respective messenger RNA and for assaying changes of these messenger RNAs during differentiation in the teratomas and for in vitro differentiation in tissue culture. We hope to test various growth and differentiation factors, such as glial maturation factor and fibroblast growth factor, on the in vitro culture system.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: An expanded knowledge of how normal and cancer cells function can hopefully be used to apply to the more practical aspects of curing disease and evaluating the effect of the environmental factors on human health. By quantitating RNA sequence expression (total, nuclear, and cytoplasmic), we may get a better feel for the information flow from the genetic material. By comparing this genetic

information flow from cells in different states of differentiation, we may gain additional insight into the molecular mechanisms underlying gene expressions during differentiation. We will then gain some knowledge into the underlying causes of cancer and environmental damage to various organ systems.

PUBLICATIONS

Harris, S. E.: Basic mechanisms of ovarian function germ cells. Env. Health Persp. (in press).

Carter, D. B. and Harris, S. E.: Chromosomal proteins from the embryoid body/teratocarcinoma system. Dev. Biol. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70047-02 LET						
PERIOD COVERED October 1, 1977 to September 30, 1978								
TITLE OF PROJECT (80 characters or less) Regulation of Gene Expression during Differentiation of Teratocarcinoma Cells								
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT								
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: D. B. Carter</td> <td style="width: 33%;">Staff Fellow</td> <td style="width: 33%;">LET NIEHS</td> </tr> <tr> <td>OTHER: S. E. Harris</td> <td>Senior Staff Fellow</td> <td>LET NIEHS</td> </tr> </table>			PI: D. B. Carter	Staff Fellow	LET NIEHS	OTHER: S. E. Harris	Senior Staff Fellow	LET NIEHS
PI: D. B. Carter	Staff Fellow	LET NIEHS						
OTHER: S. E. Harris	Senior Staff Fellow	LET NIEHS						
COOPERATING UNITS (if any)								
None								
LAB/BRANCH Laboratory of Environmental Toxicology								
SECTION Molecular Embryology Group								
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709								
TOTAL MANYEARS: 1	PROFESSIONAL: 1	OTHER:						
CHECK APPROPRIATE BOX(ES)								
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER								
☐ (a1) MINORS ☐ (a2) INTERVIEWS								
SUMMARY OF WORK (200 words or less - underline keywords) The protein-mapping technique of O'Farrell has been used to study the synthesis of total SDS soluble, soluble cytoplasmic and 5M urea extractable nuclear proteins from embryoid bodies and their derived teratoma tumors. Proteins synthesized within a four-hour labeling period and incorporating methionine- ³⁵ S show reproducible qualitative changes during growth from embryoid bodies to small (<2mm) tumors. Approximately 165 spots are visible from the soluble cytosol proteins of embryoid bodies compared to 145 spots from the corresponding fraction from teratoma tumors. There appears to be about 80% homology between the detectable proteins of embryoid body and small tumors. Alpha-fetoprotein appears to be synthesized in both the embryoid bodies and small teratomas thus far examined. The embryoid body nuclear proteins contain 146 detectable proteins whereas the teratoma contains 126 detectable proteins on 2-D gels. However, several major nuclear proteins appear in the teratoma which are not detectable in embryoid body. The total SDS extracts showed the deletion of three major proteins from embryoid bodies and the expression of three new major proteins in the small teratoma.								

PROJECT DESCRIPTION

METHODS EMPLOYED: Incubation of fresh embryoid bodies and teratoma slices in ^{35}S -methionine supplemented DMEM, preparation of 100,000 xg supernatant and purification of nuclei, extraction of nuclei with 5 M urea-50 mM Na-PO_4 , pH 7.6, isoelectric focusing in pH 4 to 7 gradients, SDS slab electrophoresis and fluorography.

MAJOR FINDINGS AND PROPOSED COURSE: The high-resolution two dimensional analysis of proteins from the embryoid body/teratocarcinoma system shows approximately 40 detectable changes in protein spots. Overall, approximately 350 proteins are resolved from cytosol and nuclear fractions. Higher specific activities are desirable for increased resolution. With such protein markers as alpha-fetoprotein, glial fibrillar acidic protein, S-100 protein and protein 14-3-2, it is possible to follow the process of differentiation in the embryoid body/teratocarcinoma system with two-dimensional gel analysis.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The teratocarcinoma system has been described as a model system for the study of early embryogenesis and neoplasia. As a model of early embryogenesis the system can provide amounts of tissue far surpassing amounts available from mammalian embryos. The OTT-6050 line which we use gives rise predominantly to neuroepithelial derivatives. These differentiated tissues in tumors have been shown to produce a number of brain specific proteins providing protein markers of differentiation. It is thus conceivable that an *in vitro* system for testing the effects of potential teratogens on differentiation in neurological tissues may arise from manipulation of the OTT-6050 teratocarcinoma line. Protein-mapping would become one of the major tools available for detection of changes in structural molecules or enzymes present in large/or small amounts which occur due to interaction with teratogenic molecules.

PUBLICATIONS

Carter, D. B. and Harris, S. E.: Chromosomal proteins from the embryoid body/teratocarcinoma system. Dev. Biol. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70049-02 LET
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Development of <u>In Vitro</u> Translation Systems for Specific Messenger RNA's and for Total Poly(A)-containing RNA Preparations		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: OTHER:	A. B. Silverberg S. E. Harris	Staff Associate Senior Staff Fellow
		LET NIEHS LET NIEHS
COOPERATING UNITS (if any)		
None		
LAB/BRANCH Laboratory of Environmental Toxicology		
SECTION Molecular Embryology Group		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 3	PROFESSIONAL: 2	OTHER: 1
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline key words) The <u>in vivo</u> mouse <u>embryoid body/teratocarcinoma</u> system is being utilized as a model to study the <u>molecular mechanisms</u> of cellular determination and differentiation and the effects of environmental agents on differentiating mammalian cells. The wheat germ S-30 and nuclease-treated rabbit reticulocyte lysate <u>in vitro</u> translation systems permit the analysis of the patterns of protein synthesis during the differentiation process from simple embryoid bodies to teratomas. <u>In vitro</u> translation systems aid in the characterization of total or polysomal poly-(A)-containing mRNA by analysis of synthesized products. Isolation of specific mRNA's (e.g. <u>alpha-fetoprotein [AFP]</u> , S-100, and glial fibrillar acidic protein [GFAP]) will permit the analysis of gene transcription with cDNA probes during differentiation and the analysis of precursors of the final protein products. AFP is produced in increased quantities following partial hepatectomy, toxic injury to the liver, and hepatomas and teratomas. <u>In vitro</u> translation of mRNA's and <u>nucleic acid hybridization</u> with a specific cDNA to AFP mRNA will permit the study of the molecular events surrounding the expression of AFP during toxic injury and carcinogenesis.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Lysates of wheat germ and rabbit reticulocytes will be used to translate mRNA in vitro. One dimensional polyacrylamide gel electrophoresis and two dimensional O'Farrell gels will be used to analyze the protein products of translation. Scintillation fluorography will be used to detect the proteins in the one- and two-dimensional gels. Immunoprecipitation will be used to detect specific protein synthesis following translation. Immunofluorescence will be used to detect specific proteins during in vitro culture or following in vivo development of embryoid bodies and teratomas. Tissue culture will be used to study the in vitro differentiation and determination of embryoid bodies and teratomas.

MAJOR FINDINGS AND PROPOSED COURSE: We have completed an analysis of the in vivo patterns of protein synthesis in simple embryoid bodies and teratomas. The major differences appear to involve alpha-fetoprotein (AFP) glial fibrillar acidic protein (GFAP) and S-100 (the latter two are glial and neural specific proteins, respectively). All three proteins appear to be made by the teratomas, but only AFP is made by the embryoid bodies and in far smaller quantities.

We have completed the purification of AFP from rat amniotic fluid and S-100 from beef brain and successfully induced production in rabbits of specific antibodies to the two proteins. We have partially purified GFAP and plan to complete the purification of this protein and start antibody production in rabbits also.

We have modified the wheat germ S-30 in vitro translation system to optimize for the synthesis of large protein products. We have begun the study of the patterns of proteins synthesized during in vitro translation of total poly(A)-containing mRNA from embryoid bodies, teratomas, fetal and adult rat livers and TCDD treated adult rat livers. Using the rabbit anti-AFP. We are examining for specific synthesis of AFP during in vitro translation with the above mRNA's.

We plan to utilize the in vitro translation system in the purification of specific mRNA's (esp. AFP mRNA). A radioactive cDNA to AFP mRNA would be a sensitive, specific probe for the expression of AFP during development, toxicity and carcinogenesis.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The research is important to the understanding of the molecular events during differentiation, toxicity (environmental or other) and carcinogenesis. An in vitro translation system is essential in the purification of specific mRNA's that may characterize states of differentiation or toxicity. Pure mRNA's can then be utilized to study gene expression in normal and abnormal states. In vitro translation is also a sensitive assay system for the expression of specific protein(s) than may characterize disease states.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70060-05 LET
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Effect of Prenatal Exposure to Foreign Chemicals on Genital Tract Function		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: OTHER:	J. A. McLachlan K. S. Korach Y. Suzuki S. E. Harris L. Levy M. Hogan M. Luster J. C. Lamb, IV	Developmental Toxicologist Staff Fellow Visiting Fellow Staff Fellow Research Chemist Mathematical Statistician Immunologist Developmental Pathologist
		LET NIEHS LET NIEHS LET NIEHS LET NIEHS EBCB NIEHS BB NIEHS EBCB NIEHS LET NIEHS
COOPERATING UNITS (if any) Bowman-Gray School of Medicine Environmental Biology and Chemistry Branch, NIEHS Environmental Biometry Branch, NIEHS		Univ. Colorado Medical Center Cancer Research Center, UNC University of Wurzburg, Wurzburg Germany
LAB/BRANCH Laboratory of Environmental Toxicology		
SECTION Transplacental Toxicology Group		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 3.6	PROFESSIONAL: 1.8	OTHER: 1.8
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The long range goals of this project are: (1) to evaluate the effects of <u>prenatal exposure to environmental chemicals</u> on the subsequent <u>reproductive capacity</u> of the offspring; (2) to investigate the mechanisms involved in the production of <u>subfertility</u> in mammals as a result of their <u>in utero exposure to foreign chemicals</u> ; (3) to assess the <u>transplacental carcinogenic potential</u> of these compounds; (4) to study the <u>physiologic disposition and metabolism</u> of these compounds in the pregnant animal and fetus; (5) to study <u>chemico-biological interactions</u> of transplacental toxicants, with special emphasis on <u>structure-activity relationships</u> ; (6) to determine if prenatal exposure to environmental agents can alter the <u>cytoplasmic receptors for steroid hormones in reproductive tract tissues</u> ; (7) to develop and utilize <u>organ culture systems</u> to study the effects of environmental chemicals on the <u>fetal ovary and reproductive tract in vitro</u> ; and (8) to evaluate the above animal models as predictors of human response. Special attention is given to <u>diethylstilbestrol (DES)</u> .		

PROJECT DESCRIPTION

METHODS EMPLOYED: The most sensitive measurement of female gonadal function is her total reproductive capacity as determined by repetitive forced breeding techniques. In order to assess the function status of the female gonad, these techniques were coupled with the determination of ovarian periodicity, quantity, and morphology of ova obtained from the female reproductive determinations of the various follicular classes. Biochemical, physiologic, and morphologic procedures were used which included polyacrylamide gel electrophoresis, radio-immunoassay, histochemistry, autoradiography, inverse isotope dilution analysis, microdissection, and scanning electron microscopy. Organ culture techniques were developed to maintain explants of fetal ovaries and reproductive tracts.

MAJOR FINDINGS AND PROPOSED COURSE: The synthetic estrogen, diethylstilbestrol (DES), is a common environmental chemical currently used as a livestock growth promotor and gynecologic medication. Experiments in our laboratory have demonstrated that prenatal exposure to DES adversely affects the reproductive capacity of the male and female offspring. Continuation of these studies has shown that such prenatal exposure results in a low incidence of female genital tract neoplasms including vaginal and uterine adenocarcinoma, well differentiated squamous cell cancers of the cervix and ovarian tumors. Stromal hyperplasia of the vagina, cervix and uterus has raised the question of the role of this tissue component in the observed lesions; the demonstration of a uterine stromal sarcoma in a prenatally DES-treated mouse further emphasizes the importance of studies on stromal-epithelial interactions during abnormal development of the genital tract. Similar lesions could not be produced following prenatal exposure to the steroidal estrogen, 17 β -estradiol. Differential fetal protein binding between DES and estradiol may help explain these results.

Scanning electron microscopy (SEM) of the genital tract of these females has revealed surface ultrastructural abnormalities which may precede neoplastic changes; SEM may provide a tool for early detection of neoplastic change in the affected tissues. This technique was also used to demonstrate hormone-related changes in genital tract tissue during the estrous cycle and with the onset of puberty. The latter study suggests that hormone responsiveness is manifested earlier in the vagina than in the uterus. These studies are being correlated with observations on the steroid receptors in the affected tissues. Additional observations on the SEM changes induced in the genital tract of ovariectomized female mice by estrogenic, progestenic and androgenic hormones were conducted to complete the baseline studies necessary to evaluate pathologic changes in the SEM appearance of the genital tract.

In continuation of studies with male offspring from DES treated mice, the fate of Mullerian duct remnants in DES treated males was determined and the role of these tissues in prostatic lesions evaluated. Mullerian remnants were observed in treated male offspring at any stage of development. Alterations

in these tissues were, in part, related to changes in estradiol and Mullerian inhibiting Factor (MIF). Additionally, SEM studies of the male genital tract have been undertaken to establish sensitive morphological parameters of cellular abnormalities. Most abnormalities observed in the genital tracts of exposed men and women have been duplicated in our mouse model. Additional alterations seen in the mouse may be predictive for the human. In both male and female offspring, attempts will be made to establish biochemical/morphological markers for genital tract lesions.

The distribution, metabolism and structure-activity relationships of DES in perinatal systems have continued. Oxidative metabolites of DES (e.g. β -dienestrol and ω -hydroxy DES) were identified in the mouse fetus and neonate exposed to ^{14}C -DES. Moreover, studies on the bioactivation of DES have been started; initial studies have shown the non-extractable binding of radioactivity to DNA and protein after incubation of ^{14}C -DES with several activating systems. The estrogenic activities of a series of DES metabolites and analogs were determined. Results suggest that DES metabolism follows alternative pathways resulting in metabolites which retain estrogenicity or those in which such activity is absent. These studies will be expanded with special emphasis on the biological significance of potentially activated metabolites; such data should aid in generalizations to other classes of estrogenic environmental chemicals. In addition, DES metabolism in target/non-target tissues and in cell culture is being evaluated. For example, it was shown that peroxidase, an enzyme inducible in estrogen target tissue, is able to metabolize DES to its major metabolite, β -dienestrol.

Studies in organ culture have shown that DES can alter normal differentiation of the genital tract in vitro. These studies will be continued to evaluate the role of organ/organ and cell/cell interactions in genital tract development. Thus, DES has been shown to alter the action of MIF on the in vitro differentiation of the Mullerian ducts. Also, experiments utilizing the separation and recombination of stroma and epithelium of DES treated fetal reproductive tracts have been undertaken to determine the role of such tissue interactions in DES induced genital abnormalities.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Although many compounds are continuously introduced into our environment, few of them have been examined for their potentially toxic effect on reproduction and development. Virtually nothing is known about the effect of prenatal exposure to common drugs and chemicals on the postnatal development of the offspring. The fact that no division of oocytes occurs postnatally in man or laboratory rodents makes the process of oogenesis especially susceptible to chemical intervention during the prenatal period. However, the effects of such in utero drug exposure may not become evident until much later in the animal's life when sexual maturity is reached. Given the possibility of long-term genetic damage to the developing oocyte or transplacental carcinogenetic

changes in the reproductive tract, reduced fertility in the offspring may be only the most obvious consequence of prenatal exposure to environmental chemicals.

PUBLICATIONS

McLachlan, J. A.: Male as well as female mice are affected by in utero exposure to diethylstilbestrol. Proceedings of Conference on Women and the Workplace, Society for Occupational and Environmental Health. Eula Bingham (Ed.): Society of Occupational and Environmental Health. Washington, D.C., 1977, pp. 32-39.

McLachlan, J. A.: Transplacental effects of diethylstilbestrol in mice. Proceedings of the Conference on Perinatal Carcinogenesis, NIC Monograph Series. (in press).

Korach, K. S., Metzler, M. and McLachlan, J. A.: Estrogenic activity in vivo and in vitro of some diethylstilbestrol metabolites and analogs. Proc. Nat. Acad. Sci. 75: 468-471, 1978.

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Metzler, M. and McLachlan, J. A.: Is diethylstilbestrol bioactivated by peroxidase mediated oxidation. J. Env. Path. Toxicol. (in press).

Metzler, M. and McLachlan, J. A.: Oxidative metabolites of diethylstilbestrol in the fetal, neonatal and adult mouse. Biochem. Pharmacol. (in press).

Luster, M. I., Faith, R. E. and McLachlan, J. A.: Modulation of the antibody response following in utero exposure to diethylstilbestrol. Bull. Environ. Contam. Toxicol. (in press).

Lamb, J. C., IV, Newbold, R. R., Stumpf, W. E. and McLachlan, J. A.: Transitional changes in the surface epithelium of the cycling mouse vagina, cervix and uterus: Scanning electron microscopic studies. Biol. Rep. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70065-02 LET
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) The Role of Chemical-Receptor Interactions in Reproduction and Transplacental Toxicity		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: OTHER:	K. S. Korach J. A. McLachlan J. Kao J. K. Haseman L. Levy	Staff Fellow Developmental Toxicologist Visiting Fellow Statistician Chemist LET NIEHS LET NIEHS LET NIEHS BB NIEHS EBCB NIEHS
COOPERATING UNITS (if any) University of Wurzburg Biometry Branch, NIEHS Environmental Biology and Chemistry Branch, NIEHS		
LAB/BRANCH Laboratory of Environmental Toxicology		
SECTION Transplacental Toxicology Group		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, N. C. 27709		
TOTAL MANYEARS: 2.7	PROFESSIONAL: 1.3	OTHER: 1.4
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The main objectives of this project are to determine whether <u>DES</u> is metabolized to biologically and hormonally inactive metabolites; to test the hypothesis that certain chemicals are "transplacental toxicants" due to their relative binding to plasma/receptor proteins particularly <u>alpha-fetoprotein</u> ; to investigate some of the biochemical mechanisms which contribute to results of prenatal exposure of hormonally active environmental chemicals in the mouse; to determine the molecular locus of transplacental toxicity using structure-function relationships of different environmental chemicals, and to determine a biochemical marker for transplacental toxicity. These objectives are being studied by using refined biochemical techniques of <u>hormone receptors</u> and <u>hormone action</u> . The basic <u>physiological effects on hormone synthesis and hormone levels</u> will be studied using <u>chemical extraction techniques and radioimmunoassays</u> . Experiments to determine the <u>carcinogenic nature of hormonally active environmental chemicals</u> will be studied <u>in vivo</u> .		

PROJECT DESCRIPTION

METHODS EMPLOYED: Hormonal effects were studied with receptor binding techniques including saturation binding, competition studies, Scatchard plot analysis, sucrose gradient centrifugation, glucose oxidation/utilization, polyacrylamide gel electrophoresis, dual isotope labeling and protamine sulfate precipitation. Biochemical studies employed spectrophotometric enzyme assays and routine chemical isolation and extraction techniques.

MAJOR FINDINGS AND PROPOSED COURSE: Female offspring given prenatal injections of DES result in two distinct groups: those with hormonally nonresponsive uteri and those that are hyperstimulated. To understand why some uteri were not hormonally responsive, we first examined the concentration of estrogen receptors in these animals. Our characterization of uterine nuclear estrogen receptors in the mouse has indicated two forms of the receptor (analogous to Type I, II reported in rat). Presently, we are determining the best experimental conditions for studying these receptor forms. Results from our laboratory have shown that those animals in the non-responsive group have significantly lower levels of estrogen receptor than controls. In order to distinguish the age at which this difference in receptor level occurs between DES treated and control animals, we have measured receptor levels in 5 through 40 day old animals. These studies suggest that levels at 5 days of age already show this pattern of receptor differences. Cytosol receptor concentrations in vaginal tissue were not significantly different from controls. Further experiments with the uterus and vagina from DES exposed mice will be continued to determine which step in the mechanism of hormone action is altered. The difference in cytosol receptor levels could not be explained by differential accumulation of receptors in the nucleus since assays of nuclear receptor in these same tissues showed no differences.

Receptor differences in control and DES treated offspring were also found in studies demonstrating the responsiveness of the receptors to estrogen administration. We have found that the mouse uterus possesses a second translocation of hormone receptor complex to the nucleus after exposure to hormone. Compounds with poor estrogenic potency lack the ability to elicit this second nuclear peak. We are presently involved with determining the role of this event in estrogen action in the mouse reproductive tract, with particular regard to the actions of hormonally active environmental chemicals, since DES treated animals gave an altered pattern of receptor depletion/replenishment as compared with untreated animals. We are now investigating receptor resynthesis, RNA polymerase activities and glucose oxidation/utilization.

In order to determine whether the metabolism of DES resulted in biologically active or inactive metabolites, certain DES metabolites and analogs were tested for estrogenic activity using both an in vivo bioassay and an in vitro receptor binding assay. Results of these studies showed good correlation between the biochemical and bioassay data. Compounds such as DES-epoxide or catechol-DES were associated with reasonable receptor binding and biological activity;

while certain metabolites, eg. β -dienestrol or w-hydroxy dienestrol, showed weak receptor interactions and poor estrogenicity. This indicates that the metabolism of DES does not result in complete inactivation of the compound. The exception to these results was indanyl-DES, a DES analog and possible metabolite, which shows strong receptor binding comparable to DES, but 20-25 times less biological activity. Receptor and plasma binding activities particularly to alpha-fetoprotein of various DES analogs and metabolites will be continued to determine the structural site of chemicals exhibiting hormonal and/or carcinogenic actions.

Binding studies with different environmental chemicals are being expanded to allow us to determine their potential hormonal activity in the model. Complete hormone action involves the ability of the hormone to influence resynthesis of its receptor; many of these DES compounds showed this hormonal property while others demonstrated some deficiency. Their ability to demonstrate this property was related to their biological efficacy.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The association of in utero DES exposure and reproductive tract cancer in human subjects is well documented. Recent development of a mouse model to study these same effects will allow us to investigate this problem more fully in the laboratory. The objectives of this project are to investigate the role of receptor protein-chemical interactions and the biochemical mechanisms associated with the toxicologic responses observed in the reproductive tract following in utero exposure to hormonally active environmental chemicals.

Since our knowledge of environmental chemical effects in utero on the reproductive system of the offspring is so limited, our studies will increase our recognition of other clinical and biomedical problems which may arise from exposure to environmental compounds. Determining the mechanism by which these chemicals may act may help in the development of safeguards against continued exposure.

PUBLICATIONS

Korach, K. S., Metzler, M. and McLachlan, J. A.: Estrogenic activity in vivo and in vitro of some diethylstilbestrol metabolites and analogs. Proc. Nat'l. Acad. Sci. 75 (1): 468-471, 1978.

Korach, K. S.: Depletion and replenishment patterns of estrogen receptors in the mouse uterus. Fourth NCTR Hormone Research Symposium. J. Tox. Environ. Health. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70068-01 LET
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Correlative Morphological Studies of Abnormal Cell Differentiation		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: OTHER:	J. C. Lamb, IV J. A. McLachlan Y. Suzuki S. E. Harris A. S. Silverberg	Developmental Pathologist Developmental Toxicologist Visiting Fellow Senior Staff Fellow Staff Associate
		LET NIEHS LET NIEHS LET NIEHS LET NIEHS LET NIEHS
COOPERATING UNITS (if any) Bowman Gray School of Medicine U.N.C. Cancer Research Center		
LAB/BRANCH Laboratory of Environmental Toxicology		
SECTION Transplacental Toxicology Group		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, N. C. 27709		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES)		
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER		
☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) These studies are designed to: (1) evaluate the influence of <u>in utero</u> exposure to environmental toxicants (for example, <u>Diethylstilbestrol</u>) on <u>fine structure</u> of the male and female reproductive tracts; (2) study the influence of environmental chemicals on <u>cell differentiation</u> and <u>cell surface ultrastructural features</u> ; (3) develop and utilize models which will determine the cell surface features associated with <u>preneoplastic</u> and <u>neoplastic transformation</u> by <u>scanning and transmission electron microscopy</u> and <u>correlative histology</u> , and (4) employ <u>histochemistry</u> to identify and <u>localize morphological markers</u> to assess the cellular or subcellular responsiveness to <u>foreign agents</u> .		

PROJECT DESCRIPTION

METHODS EMPLOYED: The scanning electron microscope has proven to be a very sensitive tool for the evaluation of cell surface changes associated with normal and abnormal cell differentiation. The pathological evaluation of lesions found with the scanning electron microscope in reproductive tract tissue is made by subsequent processing for light microscopy so that classic morphological criteria may be used to classify genital tract abnormalities. When in vitro systems such as tissue culture specimens are evaluated, the samples are first studied by phase contrast microscopy, interference contrast microscopy or immunofluorescence microscopy, and cell surface changes can be correlated to histological changes on a cell-to-cell basis. Correlative or concurrent transmission electron microscopy or histochemistry has also been employed. Histochemical methods have also been developed as highly sensitive assays to be used in conjunction with biochemical or enzymological assays.

MAJOR FINDINGS AND PROPOSED COURSE: Previous studies in our laboratory have demonstrated that in utero exposure of the CD-1 mouse to diethylstilbestrol (DES) may serve as a model for prenatal toxicity. Prenatal exposure DES is associated with male and female genital tract abnormalities in mice.

Studies in our laboratory have demonstrated that scanning electron microscopy (SEM) may provide a tool for the detection of early neoplastic changes. The surface ultrastructural features of the lumen of the female mouse genital tract was evaluated during the estrous cycle and during development in normal CD-1 mice. The hormone-dependence of fine structural features of the cell surface was demonstrated in ovariectomized, hormone-treated females where various characteristics of intact animals were experimentally induced. Subsequent studies on DES-treated mice indicate that cell surface features are directly related to abnormal cell differentiation. Changes in the cell surface are correlated with alterations in the histological features of DES-exposed offspring. Histochemical and high resolution electron microscopic studies also play an integral role in the evaluation of the mechanism of DES-toxicity. Enzyme histochemical studies are ongoing to determine the precise location of the metabolic enzymes, peroxidase, which may activate DES to a toxic intermediate.

We are also evaluating cell surface changes associated with cell differentiation in the OTT 6050 teratoma cell line. Cell surface features are directly compared to the appearance of the same cells by phase contrast microscopy. Immunofluorescence and immunohistochemical studies to isolate cells with antigenic markers of cell differentiation are now being attempted to identify SEM markers of cell differentiation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Cell surface changes are thought to be important in neoplastic transformation. However, the influence of in utero exposure to environmental toxicants on the

subsequent development of cell surface ultrastructure has not been evaluated. The transplacental toxicity of DES in the CD-1 mouse is being used as a model for morphological changes associated with exposure to toxic chemicals early in development. Neoplasia may be the endpoint of abnormal differentiation or altered development in these animals. Comprehensive investigations into normal differentiation employ in vitro systems and evaluate morphological changes in normal development and ageing, thereby establishing a baseline from which we can evaluate pathological lesions.

PUBLICATIONS

Lamb, J. C., IV, Newbold, R. R., and McLachlan, J. A.: Modulation of the female genital tract by hormones. SEM, 2: 773-778, 1978.

Lamb, J. C. IV, Newbold, R. R., Stumpf, W. E. and McLachlan, J. A.: Transitional changes in the surface epithelium of the cycling mouse vagina, cervix, and uterus: Scanning electron microscopic studies, Biol. Rep., 1977 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70070-02 LET
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Transplacental Pathology of Environmental Chemicals		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: OTHER:	M. A. Peters J. Kao R. L. Dixon E. E. McConnell	Visiting Scientist Visiting Fellow Laboratory Chief Pathologist LET NIEHS LET NIEHS LET NIEHS EBCB NIEHS
COOPERATING UNITS (if any) Environmental Biology and Chemistry Branch Division of Laboratory of Animal Resources, Duke		
LAB/BRANCH Laboratory of Environmental Toxicology		
SECTION Developmental Pathology Group		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 3	PROFESSIONAL: 1	OTHER: 2
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) This project is designed to evaluate the effect of environmental chemicals on offspring of female rats subjected to <u>totigestational exposure</u> . Selected biochemical, histological and behavioral parameters are monitored for <u>transplacental pathology</u> . To date studies on three chemicals have been initiated: <u>methyl-n-butyl ketone</u> , <u>enflurane</u> (ethrane), and <u>ethchlorvynol</u> (placidyl). Although some behavioral and physiological changes have been observed, no definite conclusions are possible as yet.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Mated female Fischer rats are exposed to selected chemicals throughout gestation by appropriate routes beginning on day 0 of gestation and continuing through day 20. A dose causing minimal overt effects at birth is used. Selected physiologic, biochemical, histological and behavioral observations are made at each of five stages of development: newborn, weanling, puberty, five months and 18 months of age. The organ systems monitored include: liver, kidney, hematopoietic, sensory, central and peripheral nervous system, reproductive, skeletal muscle, immunological, cardiovascular, etc. Standard techniques and assay procedures are employed.

MAJOR FINDINGS AND PROPOSED COURSE: To date totigestational studies on three chemicals have been initiated.

(A.) Totigestational toxicity of methyl-n-butyl ketone (MBK). When administered by inhalation, at 500 ppm the only effect of MBK noted was a 12 to 24 hour delay in parturition. At 1000 ppm the exposed dams were smaller than corresponding controls which correlated with a decrease in litter size. While birth weight of treated and control animals was comparable, the growth rate of offspring from treated females was decreased at all stages of development to adulthood. Female offspring of treated females showed retarded sexual maturation as determined by time of vaginal opening. Shock avoidance acquisition and retention were deficient in female offspring but not in males. Female offspring of treated animals were significantly more active when tested on an activity wheel. No other lesions have been detected grossly, histologically or biochemically.

Females exposed to 200 ppm MBK had a reduced food intake but an elevated water intake relative to controls. The decreased food intake was reflected in a decreased weight gain. Normal offsprings were delivered at parturition. However, growth rate of offspring were decreased at all stages of development including adulthood. Behavioral observations from the inclined screen test showed an overall effect in the weanling offsprings from treated animals, but the effect was not marked enough to be declared significant on a pair wise basis. Significant overall treatment effects on behavior were also observed at puberty. Clinical chemistry determinations revealed no significant differences between offsprings from treated female and offsprings from control animals.

(B.) Enflurane (Ethrane). Enflurane was administered by inhalation at 0.15% for six hours per day for 21 days starting at day 0 gestation. Serum fluoride was monitored for three consecutive days, day 0, 1 and 2. Extrapolation from these results suggest that a plateau level of approximately $1.7 \times 10^{-5}M$ would be reached by day 5 or 6. Enflurane treated females delivered a larger

percentage of small litters (less than seven pups) than did controls. Birth weight and subsequent growth rate of offspring of treated females were comparable to controls. A variety of behavioral tests showed no significant differences between young treated and control females. Clinical chemistry tests in weanling pups of treated females revealed an increased creatinine phosphokinase activity and elevated triglyceride level. At puberty and at the adult stage, behavioral tests showed no significant differences between offspring from treated and control females. The differences in clinical chemistry observed in weanling pups were not evident at pubertal or adult stages. However, triglyceride levels were slightly decreased in pubertal pups from treated females when compared to controls. All other parameters were comparable to controls.

(C.) Ethchlorvynol (Placidyl). Ethchlorvynol was administered daily by oral intubation throughout gestation at 6 doses ranging from 0 (olive oil) to 80 mg/kg. No alteration in maternal weight or fetal development was apparent. Clinical chemistry determinations in weanling animals revealed an elevated blood glucose and a decrease in phosphorus, creatine phosphokinase, SGOT, SGPT and in $(\text{Na}-[\text{Cl} + \text{CO}_2])$ balance. No differences in behavior have been observed. At puberty female offsprings from treated animals showed a significant increase in phenobarbital sleeping time, but only at the low treatment dose. Male offsprings showed little or no effects. Clinical chemistry determination in puberty pups from treated animals revealed an elevated blood glucose and a decreased BUN at 20 mg/kg dose. Triglycerides were elevated at the 80 mg/kg dose. Creatine phosphatase was significantly decreased by the 20 mg/kg dose and sodium balance was decreased at the 80 mg/kg dose.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The human population now has a lifetime exposure to an increasingly large number of chemicals with incompletely defined biological activity. Among the least adequately understood characteristics of many of these chemicals are their potential toxicity to man at his most vulnerable stage of development--conception to birth. While the more immediate transplacental effects such as abortion or teratogenic changes are relatively well studied among these agents, the often more insidious and potentially more harmful lifetime effects have received little attention. The great difficulty in correctly ascribing gestational origin to lesions in the human population which are subtle or do not become apparent until later in life makes laboratory and epidemiological investigations in this area essential. Hopefully on-going studies will yield data which can be used to identify chemicals in the environment which produce adverse transplacental changes at levels to which the human population is exposed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70080-05 LET																														
PERIOD COVERED October 1, 1977 to September 30, 1978																																
TITLE OF PROJECT (80 characters or less) The Study of Toxic Effects of Environmental Chemicals on Spermatogenesis																																
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																																
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 45%;">I. P. Lee</td> <td style="width: 20%;">Pharmacologist</td> <td style="width: 10%;">LET</td> <td style="width: 10%;">NIEHS</td> </tr> <tr> <td>OTHER:</td> <td>R. L. Dixon</td> <td>Laboratory Chief</td> <td>LET</td> <td>NIEHS</td> </tr> <tr> <td></td> <td>K. Suzuki</td> <td>Visiting Associate</td> <td>LET</td> <td>NIEHS</td> </tr> <tr> <td></td> <td>R. Shoji</td> <td>Visiting Associate</td> <td>LET</td> <td>NIEHS</td> </tr> <tr> <td></td> <td>H. Mukhtar</td> <td>Visiting Associate</td> <td>LP</td> <td>NIEHS</td> </tr> <tr> <td></td> <td>J. Bend</td> <td>Visiting Scientist</td> <td>LP</td> <td>NIEHS</td> </tr> </table>			PI:	I. P. Lee	Pharmacologist	LET	NIEHS	OTHER:	R. L. Dixon	Laboratory Chief	LET	NIEHS		K. Suzuki	Visiting Associate	LET	NIEHS		R. Shoji	Visiting Associate	LET	NIEHS		H. Mukhtar	Visiting Associate	LP	NIEHS		J. Bend	Visiting Scientist	LP	NIEHS
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TOTAL MANYEARS: 4.3	PROFESSIONAL: 4	OTHER: 0.3																														
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☐ (a1) MINORS ☐ (a2) INTERVIEWS																																
SUMMARY OF WORK (200 words or less - underline keywords) These studies seek to assess the effects of environmental agents on <u>spermatogenesis</u> . Agents are tested, test systems validated and new approaches to toxicity testing proposed: (1) examine germ cell repair and <u>DNA synthesis</u> as indicators for chemical <u>mutagenesis</u> ; (2) utilize an integrated approach to study toxic effects on <u>reproductive function</u> ; and (3) investigate the <u>toxication-detoxication</u> of <u>polycyclic hydrocarbons</u> in gonadal tissue. To date, selected mutagens, trace metals, TCDD, N ₂ O, Freon 22 and DBCP have been studied. A new sensitive alkaline elution analysis of germ cell DNA appears useful as a predictor of environmental agents which damage DNA. A DNA repair deficient mouse strain has been sought. Biochemical and toxic effects of environmental agents on rodent spermatogenesis are also studied by an integrated approach using spermatogenic "marker" enzymes, plasma FSH, LH, testosterone, histologic changes, and the <u>in vitro</u> perfusion of <u>testis</u> with selected environmental chemicals. These biochemical and toxic effects were correlated to functionality of spermatozoa through <u>serial mating</u> studies. These studies contribute to an improved understanding of mechanism to toxicity, the reliability of laboratory animal toxicity tests for mutagenic and reproductive effects and validity of their extrapolation to humans.																																

PROJECT DESCRIPTION

METHODS EMPLOYED: (A) In vivo DNA repair was studied by labeling spermatogonial DNA with repeated i.p. administration of tritiated thymidine to 15 to 20 day old rats. Test mutagens were administered and seminiferous tubules were isolated from tubules by 0.1% collagenase digestion. Elution profiles defining DNA molecular size were obtained by alkaline elution analysis. In vitro and in vivo DNA repair studies were accomplished by pretreating animals with mutagens, followed by tritiated thymidine in the presence of hydroxyurea. To determine comparative DNA repair synthesis in different mouse strains, the following mouse strains were used: CD-1, RF/J, LP/J, SWR/J, Nude homo/J, Nude hetero/J, C57BL/J, RIII/J, AKR/J, PL/J, SM/J ST/J and BALB/J.

(B) In vitro perfusion of rat testis. Rat testis was quickly exercised and the testicular artery was cannulated with a 80-100 micron glass capillary tube with polyethylene tubing. Oxygenated Krebs-ringer bicarbonate solution containing 3% albumin (BSA fraction IV) was used to perfuse testis at 20 ml g⁻¹ hr⁻¹. Benzopyrene and benzo(a)pyrene 4, 5-oxide-H³ at different concentrations were perfused; metabolites, benzo(a)pyrene 4, 5-diol and the glutathione conjugate of the diol were determined by combination of differential solvent extraction, thin layer chromatography and liquid scintillation spectrometric assays. Viability of perfused testis was determined by glucose consumption, concentration of glycolytic intermediates, ATP, PO₂ of both perfusate and effluent.

(C) Determination of polycyclic hydrocarbon activating and deactivating enzyme systems along with mixed function oxidase(s) system. Specifically, benzo(a)-pyrene hydroxylase, epoxide hydrase, glutathione S-transferase and cytochrome P-450 were measured in both testicular tissues as well as in prostate gland. Various factors affecting the specific activities of these enzymes in testis and prostate gland were studied [e.g. effects of hypophysectomy, pretreatment of environmental chemicals such as tetrachlorodibenzo-p-dioxin, 1,2-dibromo-3-chloropropane, 3-methylcholantrene and benzo(a)pyrene.]

(D) In vivo assessment of fertility. Fertility studies were conducted on rats exposed to the following compounds: Freon 22 and DBCP. The serial mating technique was utilized. Male animals were housed singly with a single virgin female and subjected to pretreatment serial mating for 2 weeks. Animals were treated on day 0 with test compounds or saline. The treated males were mated with single virgin females for the duration of each estrous cycle (five days); at the end of each cycle the female was replaced with new virgin females. The serial mating studies are terminated 60 days after the treatment. Nine days after the end of breeding period, when a female could be approximately 12.5 days pregnant, the female mice were sacrificed, uteri and fetuses examined and the numbers of reabsorptions and viable fetuses recorded. Fertility profiles were drawn as a function of spermatogenic cell differentiation to determine which germ cell types were affected by test chemicals.

(E) In vitro fetal organ cell aggregation patterns as a short term test for teratogens. Pregnant Sprague-Dawley rats on day 14 of gestation were exposed to a single irradiation of 25, 50, 100 and 200 R, respectively. Immediately after irradiation, fetal brains were excised and monodispersed fetal brain cells obtained by collagenase treatment (0.1% v/v). Five million cells in 3 ml of minimum essential medium with Eagle's base (pH 7.3-7.5) were cultured in a gyratory shaker (70 rpm) at 37°C for 48 hrs. The fetal brain cell aggregation patterns and cell aggregation volumes were measured and recorded at 1, 2, 4, 24 and 48 hrs. of incubation. Cell surface changes as well as the site of cell interaction were observed with scanning electron microscopy.

MAJOR FINDINGS AND PROPOSED COURSE: (A) DNA repair in spermatogenic cells of several strains of mice and CD-1 rats. Alkaline elution analysis is a new sensitive method for the measurements of DNA single strand breaks and cross-links in mammalian spermatogenic cells, based on the kinetics of alkaline elution of DNA from cell lysates. During the course of studies with various mutagens, it was found that two types of DNA damage occurred in spermatogenic cells: (1) DNA single strand breaks and (2) DNA inter- and intra-strand cross-links and/or cross-links between DNA strand and nucleoproteins. This cross-linking interaction stabilized DNA strands and DNA became resistant to alkaline elution. Furthermore, DNA reacted with polyfunctional alkylating agents such as busulfan, triethylenemelamine, mitomycin C, nitrosourea; these mutagens were found to be resistant to 500 rad X-radiation, suggesting the stabilization of DNA macromolecule against ionizing radiation. These two types of spermatogenic cell DNA damage can be readily determined and could be used for detecting environmental mutagens at mammalian germ cell level. Furthermore, it appeared that the rate of unscheduled DNA synthesis in spermatogenic cells exposed to MMS was not statistically different but the amount of unscheduled DNA synthesis was different in the following order: C57Bl/J, RF/J, Nude homo/J, RIII/AnJ, CD-1, AKR/J, PL/J, Nude hetero/J, C3H/HeJ, SWR/J, SM/J, ST/J, LP/J, BALB/cJ. C57Bl/J showed the most efficient DNA repair synthesis while BALB/cJ showed the least efficient DNA repair activities after MMS treatment. The relationship between the DNA repair synthesis activity and the degree of DNA single strand breaks were studied in order to assess whether increased DNA repair activity was due to increased DNA breaks or lower DNA repair activity was due to lower degree of DNA single strand breaks. The degree of DNA single strand breaks were not significantly different from each other. Whether these differences in DNA repair synthesis would reflect MMS induced dominant lethality in these strains of mice needs to be substantiated experimentally. Screening for suitable mouse strains will be continued and a DNA repair deficient strain will be tested for dominant lethal assays to determine the extent of mutation sensitivity and to assess its usefulness for a routine in vivo mutagen screen.

(B) Arylhydrocarbon activating and deactivating enzymes in testicular tissue. Microsomal epoxide hydrase and glutathione S-transferase (GSH-S-T) activities were determined with styrene oxide as substrate in rat testis during postnatal development. The ontogeny of these enzymes were also followed in liver for

comparison. Testes of six-day old rats had high GSH-S-T activities ($66 \text{ nmole min}^{-1} \text{ mg protein}^{-1}$) about 50% of adult levels. Transferase activity then developed slowly and reached a maximum by 165 days of age. Specific activity of GSH-S-T in testes of six-day old rats was three to four times that of liver. In contrast, epoxide hydrase activities of both liver and testes were very low in prepubertal rats, but they increased dramatically at the onset of puberty and reached maximum activities by 45 days of age. Microsomal and supernatant fractions prepared from rat spermatogenic cells had about twice the epoxide hydrase (EH) and GSH-S-T specific activities of the similar fractions from interstitial cells. On the other hand benzo(a)pyrene hydroxylase (AHH) activity and cytochrome P-450 content were at least two-fold greater in microsomal fractions from interstitial cells than those from spermatogenic cells. Further studies indicated that testicular AHH was not inducible by 3-methylcholanthrene or phenobarbital pretreatment while liver microsomes were significantly induced. In contrast, HCG (FSH and LH activity of 1 to 1 ratio) treatment to hypophysectomized rats (six weeks after surgery) induced a two to three fold increase of AHH activity in testis but not in the liver. *In vitro* perfusion of testis with tritium labeled benzopyrene 4, 5-oxide demonstrated that this active metabolite of benzopyrene is rapidly metabolized by EH and GSH-S-T. The rate of metabolism of this active metabolite by testis appeared to follow first order kinetics and were concentration dependent. GSH-S-T conjugation rate was 10 fold greater than that of EH activity. This difference further demonstrates close similarity to the cell free system. *In vitro* perfusion of rat testis will be employed to study pharmacokinetic aspects of environmental chemicals.

(C) Various environmental chemicals such as TCDD, BP, and DBCP induced significant levels of AHH and EH specific activities in rat testis and prostate gland. The highest induction of AHH activity was observed with a single dose of TCDD (10 $\mu\text{g/kg}$ body weight). A single oral dose of TCDD (10 $\mu\text{g/kg}$) induced SA of AHH and cytochrome P-448 levels in liver (L), testis (T) and prostate gland (PG) as a function of time. The maximum induction of AHH activity and cytochrome P-448 in L, T and PG were 4, 1.5 and 150 and 1.4, 1.3 and 166 times control levels, respectively. After TCDD treatment, the optimum time for maximal induction of AHH activity in L, T and PG was 48, 16 and 16 hrs., respectively: for cytochrome P-448 in L, T and PG, it was 48 hrs for each tissue. The induction of AHH and cytochrome P-448 in each of these tissues can be completely inhibited by a single pretreatment with either actinomycin-D or cycloheximide suggesting TCDD induces *de novo* synthesis of specific proteins. The huge increase in AHH activity in the PG following TCDD was particularly noteworthy. The induced AHH and cytochrome P-448 levels in PG were about 0.5 and 0.1, respectively, of that seen in normal hepatic microsomes. Thus, induction of AHH activity and cytochrome P-448 may contribute to the etiology of prostatic cancer.

Biological significance of induced AHH activity in rat testis and prostate gland will be studied in terms of quantitative relationship between DNA adduct and the AHH activity and to degree of prostatic cell transformation and tumorigenicity.

(D) DBCP has been shown to be mutagenic and carcinogenic in bacterial system and experimental animal systems. Furthermore, DBCP has also been demonstrated to induce germ cell DNA breaks. However, a single MTD of DBCP (80 mg/kg body weight; PO) failed to demonstrate dominant lethality. Freon 22 also failed to demonstrate dominant lethal effects. However, DBCP significantly induced AHH activity in rat kidney suggesting that kidney microsomes should be used for screening various halogenated compounds in Ames' test system (e.g. Many halogenated mutagens were false negative with hepatic microsomes).

(E) In search of a simplified in vitro test system to assess the teratogenic effects of both physical and chemical environmental factors, we investigated effects of total maternal body X-irradiation and subsequent changes in aggregation patterns of enzymatically isolated fetal brain cells. Knowing that total maternal body X-irradiation of day 14 of gestation results in multiple defects including microcephaly and/or hydrocephalus, we exposed pregnant Sprague-Dawley rats on day 14 of gestation to a single irradiation of 24, 50, 100 and 200 R, respectively. Within the first 4 hrs., both size and shape of the brain cell clusters from control and experimental groups were not significantly different. But, at 24 and 48 hrs., the size of cell aggregates in controls (50-1700 μ dia.) were significantly greater than those of X-irradiated animals (50-200 and 50-1000 μ dia. at the doses of 200 and 100 R, respectively). In contrast, no differences in the cell aggregation pattern were observed in the control and the experimental group receiving low doses of X-irradiation. The preliminary results suggest that the membrane factors which influence cell aggregation may be a useful parameter to assess early effects of X-irradiation induced brain deformity. Whether X-ray induced cell aggregation change can be induced by teratogens or not needs to be studied.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In recent times, the mutagenic and carcinogenic potential of environmental chemicals has become a major concern for human health. Consequently, many useful short term mutagenic test systems employing certain bacterial mutagens such as Ames test and, in addition, test systems using mammalian cell lines have become available. The currently available systems to detect mammalian germ cell mutations are the cytogenetic, dominant lethal test and a recessive specific locust assay in mice. Therefore, detection of spermatogenic cell DNA damage can be a useful tool in predicting mutagenic potential of environmental chemicals.

Elution patterns of DNA single strand breaks and stabilization of DNA and/or increased DNA repair synthesis in spermatogenic cells after chemical exposure are found to be useful as mutagenic and toxic indicator of mammalian germ cells. Studies of toxic effects of environmental agents on spermatogenesis and reproductive organs by integrated approach were found to be important in explaining the mechanism of toxic effects. Alteration of spermatogenic "Marker" enzymes was useful in early prediction of specific spermatogenic cell damage. The in vitro cell free system and in vitro perfusion of testis to study chemical activation and deactivation by interstitial and germinal compartments of

testis have provided important information with respect to polycyclic hydrocarbon activating and inactivating enzymes in germinal tissues as well as in steroidogenic cells. Furthermore, in vitro perfusion of testis provides a unique model system to study integration of molecular, biochemical and physiological data to pharmacokinetic parameters. This integrated approach for studying toxic effects of environmental chemicals on male reproductive organs may serve as an important model for the study of infertility in the human population.

PUBLICATIONS

Schmid, B., Lee, I. P., and Zbinden, G.: DNA repair process in germ cells demonstrated in ejaculated sperms of rabbits treated with methylmethane sulfonate. Arch. Toxicol. 40: 37-43, 1978.

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Lee, I. P. and Dixon, R. L.: Mutagenicity, carcinogenicity, and teratogenicity of procabazine. Mutation Res. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70085-01 LET
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Development of <u>In Vitro</u> Models for Toxicological Testing in Reproduction		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: J. L. Hall Research Physiologist LET NIEHS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Environmental Toxicology		
SECTION Germ Cell Development Group		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.0	OTHER: .5
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) These studies are designed to predict changes in the male reproductive system which are expressed as altered biochemistry (proteins) and/or function (fertility). A workable fertilization system using <u>human sperm</u> and <u>animal ova</u> will be developed as a model to test this altered fertility in humans induced by environmental agents. An <u>electrophoresis model</u> discussed in this report will complement the fertilization studies and should provide a means of reducing subtle <u>genetic and biochemical changes</u> to an easily interpretable system.		

PROJECT DESCRIPTION

METHODS EMPLOYED Assessment of the fertilizing ability of human spermatozoa requires the observation of results from natural mating or artificial insemination, neither of which are used in experimental or routine testing procedures. Nor are there in use analytical procedures permitting the quantitation of proteins and enzymes reflective of subtle changes in reproductive fluid composition. Methods for developing usable animal models for determining changes in human fertility or changes in gene products resulting from environmental factors are described as follows:

A. Female laboratory animals are superovulated to produce two to three times the normal supply of ova. Digestion of the zona pellucida of the ovum by enzymatic treatment removes the "presumed" interspecies barrier to fertilization. Human spermatozoa (fresh and frozen) from normal and suspected infertile males are obtained from a local sperm bank. Both male and female gametes are washed and placed into one culture dish and incubated under defined conditions *in vitro*. Prior to the combination of gametes, the spermatozoa are preincubated for various predetermined periods. This procedure determines the capacitation time (period for mature sperm to acquire the fertilizing ability). At the completion of a culture period, the ova are examined by light and electron microscopy for sperm penetration into the egg cytoplasm or at a later time for the formation of male and female pronuclei and subsequent cleavage. Fertilized eggs are analyzed biochemically for changes in protein composition.

B. Reproductive fluids from human subjects contributing the spermatozoa for *in vitro* fertilization and male reproductive tract fluids from laboratory animals are screened by SDS electrophoresis (Laemmli's method) or examined further by the perpendicular (two-dimensional) electrophoretic technique of Anderson. Modification of this technique to employ concave or convex exponential gradient acrylamide gels should provide the most discriminating means of analyzing *in vivo* produced proteins and genetic variants. Specific isozymes are identified with appropriate stains following separation.

MAJOR FINDINGS AND PROPOSED COURSE: Interspecies fertilization involving human sperm and laboratory animal ova has proven successful with the hamster but not with the mouse or rat. Results indicate that at least 4-6 hours of preincubation are required for human spermatozoa to attach to and penetrate the zona-free hamster egg. Following capacitation, human sperm can actually activate the hamster egg by inducing the resumption of the second maturation division and extrusion of the second polar body. Union of the human male pronucleus and hamster female pronucleus follows. Cleavage to the two-cell stage has been accomplished in a defined medium as well as in a serum supplemented medium. Almost equal success has been achieved with frozen human sperm, but improvement of the efficiency of the system is needed.

Unlike the human-hamster system, heterologous fertilization studies with mouse sperm and hamster ova have been developed to meet the criteria for a usable system to assess the influence of environmental agents on mouse sperm fertility. Rapid and efficient fertilization is accomplished with up to 100% sperm penetration in less than two hours after insemination in vitro. A homologous fertilization system of mouse spermatozoa and mouse eggs is not practical as a rapid test for fertilizing capacity since less than 10% of zona-free eggs are penetrated by mouse sperm and intact eggs require several hours before a substantial rate of penetration is observed. Evidence that heterologous ova, such as the hamster's, may provide an accurate means of detecting subnormal fertilization was provided by the lack of penetration of immature mouse sperm (from the caput epididymis) into hamster eggs.

Our objectives are to continue to work with the human-hamster system to increase the penetration efficiency and/or rates of fertilization. Ova from other species of animals are being investigated for an improved model.

Plans are to provide a comprehensive background of information on the protein characters of early embryos from heterologous or homologous crosses to compare with data obtained similarly from early embryos resulting from a parent that was exposed to environmental compounds. Initial studies with mouse eggs indicate that soluble proteins from as few as twenty eggs can be quantitatively measured following electrophoretic separation.

Experiments are planned to examine the effect of proposed male contraceptives, pesticides and alkylating agents on seminal plasma protein patterns and on sperm fertilizing capacity.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Models for testing toxic compounds and predicting mutation are needed, especially in the area of human male reproduction and early embryo development.

Combining studies of interspecies fertilization and embryo culture with sensitive electrophoretic analysis of proteins should allow the early prediction of significant increases in infertility or rates of mutation due to environmental factors.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70120-07 LET																																			
PERIOD COVERED October 1, 1977 to September 30, 1978																																					
TITLE OF PROJECT (80 characters or less) Metabolism and Binding of Steroids and Xenobiotics in Developing Systems																																					
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																																					
<table style="width: 100%; border: none;"> <tr> <td style="width: 5%; vertical-align: top;">PI:</td> <td style="width: 70%;">G. W. Lucier</td> <td style="width: 20%;">Head, Developmental Toxicology</td> <td style="width: 5%;">LET</td> <td style="width: 10%;">NIEHS</td> </tr> <tr> <td></td> <td>C. M. Schiller</td> <td>Senior Staff Fellow</td> <td>LET</td> <td>NIEHS</td> </tr> <tr> <td></td> <td>C. A. Lamartiniere</td> <td>Senior Staff Fellow</td> <td>LET</td> <td>NIEHS</td> </tr> <tr> <td></td> <td>C. S. Dieringer</td> <td>Staff Fellow</td> <td>LET</td> <td>NIEHS</td> </tr> <tr> <td></td> <td>W. Powell-Jones</td> <td>Visiting Fellow</td> <td>LET</td> <td>NIEHS</td> </tr> <tr> <td></td> <td>E. Lui</td> <td>Visiting Fellow</td> <td>LET</td> <td>NIEHS</td> </tr> <tr> <td></td> <td>H. B. Matthews</td> <td>Research Chemist</td> <td>LP</td> <td>NIEHS</td> </tr> </table>			PI:	G. W. Lucier	Head, Developmental Toxicology	LET	NIEHS		C. M. Schiller	Senior Staff Fellow	LET	NIEHS		C. A. Lamartiniere	Senior Staff Fellow	LET	NIEHS		C. S. Dieringer	Staff Fellow	LET	NIEHS		W. Powell-Jones	Visiting Fellow	LET	NIEHS		E. Lui	Visiting Fellow	LET	NIEHS		H. B. Matthews	Research Chemist	LP	NIEHS
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COOPERATING UNITS (if any) Laboratory of Pharmacology - National Institute of Environmental Health Sciences Department of Biochemistry - University of North Carolina School of Medicine																																					
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SUMMARY OF WORK (200 words or less - underline key) It is the long-range plan of this project to study <u>steroid</u> and <u>xenobiotic metabolism and binding</u> in target tissues with emphasis on <u>hormonally-active chemicals</u> . We have studied the <u>placental transfer</u> and <u>fetal uptake</u> of chemicals such as the PCBs, PBBs, cadmium, DES, and TCDD. The pharmacokinetics of these chemicals are studied in relation to possible developmental toxicity, emphasizing chemically-induced alterations in the perinatal imprinting of hepatic enzyme activity. The sequence of biochemical events leading to programmed responses in control and neonatally-treated animals are investigated so that predictions might be made regarding structure-activity relationships. The perinatal development of <u>steroid receptors</u> in the liver and brain are evaluated for their influence on the programming process. Results have characterized structure-activity relationships for the perinatal pharmacology and toxicology of pure PCB isomers.																																					

PROJECT DESCRIPTION

METHODS EMPLOYED: For the PCB and PBB perinatal pharmacology studies, ^{14}C -labeled isomers were used. Analogs investigated have been 4-chlorobiphenyl (1-CB), 4,4'-dichlorobiphenyl (2-CB), 3,4-3',4'-tetrachlorobiphenyl (4-CB), 2,3,6-2',3',6'-hexachlorobiphenyl (6-CB'), 2,4,5-2',4',5'-hexachlorobiphenyl (6-CB), and 2,4,5-2',4',5'-hexabromobiphenyl (6-BB). Metabolites were identified in some tissue samples by thin layer and gas chromatography. Rats were generally treated orally with the PCBs or PBB on the 18th day of gestation or two weeks prior to mating. The relative contributions of placental transfer and milk secretion to newborn tissue levels were evaluated by foster mother experiments. Attempts are being made to develop a pharmacokinetic model for 6-CB and 6-BB tissue distributions during the perinatal period in collaboration with LEP and BB.

The developmental toxicities of 1-CB, 4-CB, and 6-CB were studied in dose-response experiments by administering the organohalogen five times between the 8th and 18th days of gestation. Altered patterns of enzyme development were evaluated as well as histological changes. Steroid receptors in rat liver cytosol were measured by a variety of assay procedures utilizing dextran-coated charcoal, ammonium sulfate, protamine sulfate, and sucrose gradients. Total and available binding sites were measured. Binding constants and amounts of receptors were determined by Scatchard Plots. Nuclear receptors and translocation processes were studied using liver and uterine slices and cell free systems. Substrate specificities were evaluated by competitive binding techniques. Estrogenicity potentials of PCB metabolites were also determined by competitive binding. The role of the ontogeny of estrogen binding proteins in the development of estrogen responsiveness of hepatic enzymes was also studied.

The importance of the pituitary and gonads in regulating synthesis of liver enzymes and estrogen receptors was studied using intact, hypophysectomized and castrated rats. Effects of hormonally-active chemicals on the pituitary-hypothalamic-gonadal regulation of sex-dependent differences in the Cd-mediated repression of hepatic mixed-function oxidases was investigated in relation to age, dose, and hormonal influences.

Assays for UDP glucuronyltransferase, benzpyrene hydroxylase, aminopyrine demethylase, ethylmorphine demethylase, cytochrome P-450 and ethoxyresorufin-O-deethylase were conducted using standard procedures.

MAJOR FINDINGS AND PROPOSED COURSE: Although much is known about the fate of specific PCB isomers in adult animals, little information is available concerning the pharmacology of these isomers in perinatal systems. Therefore, we have studied specific fetal and newborn tissue distribution of several ^{14}C -labeled chlorinated biphenyls. Two protocols were utilized: (1) PCB or PBB isomers administered on the 18th day of gestation allowing for comparisons

of rapidly- and slowly-metabolized analogs; (2) PCB or PBB isomers administered to female rats two weeks prior to mating so that equilibration is reached between blood and adipose tissue before there is a fetal compartment. This protocol is applied to evaluation of the relative role of placental transfer and milk secretion of the biologically-persistent PCB and PBB analogs. Protocol 1 revealed that PCBs which do not accumulate significantly in adipose tissue do accumulate dramatically in fetal intestine (1-CB, 2-CB, 4-CB, and 6-CB'). Intestinal radioactivity was primarily in the form of PCB glucuronides and accumulation was dependent on the fetal onset of UDP glucuronyltransferase. Data from protocol 1 also revealed that fetal and maternal blood concentrations of 4-CB were considerably greater than that of other isomers.

Protocol 2 revealed that 6-CB (which does not accumulate in the fetal intestine) is secreted in the milk although significant newborn tissue levels of this isomer also result from placental transfer. Newborn skin and fat contained the highest concentration of 6-CB after gestational and/or milk exposure. The pronounced increase in newborn tissue concentrations observed immediately after birth is a function of 6-CB redistribution from brown fat as well as newborn exposure to this chemical during suckling. Placental transfer rates of 6-BB are considerably slower than 6-CB although milk concentrations of both isomers were equivalent. Adult rats (56 days old) which had received 6-CB or 6-BB via placental transfer during their gestations and via milk secretion from treated mothers had adipose tissue concentrations equivalent to the dam.

PCB administration to pregnant rats (non-fetotoxic dose) resulted in hepatic enzyme induction during perinatal development as measured by UDP glucuronyltransferase (UDPGT), aminopyrine demethylase (AD) and ethoxyresorufin deethylase (ERD) activities according to precise structure-activity relationships. 4-CB induced P-448 dependent enzymes (ERD) in offspring whereas 6-CB induced P-450 dependent mixed-function oxidases (AD) in preweanling animals. UDPGT, which is not P-450 dependent, was induced by both 6-CB and 4-CB. Adult male (F_1) liver UDPGT from 4-CB treated dams was significantly lower than controls whereas hepatic female UDPGT was unchanged. This suggests specific structural requirements for PCB-mediated feminization of hepatic enzymes since 6-CB did not cause these effects.

Since PCBs apparently exhibit hormonal activity in developing animals, future experiments will examine the role of chlorine position and number as a determinant of estrogenic potential of PCB isomers in developing systems. The role of metabolic hydroxylation in estrogenic action of the PCBs will also be investigated. Estrogenicity of pure PCBs and hydroxylated metabolites will be determined by competitive binding studies for the cytosolic estrogen receptor in liver and uterus and by *in vivo* bioassays. Since the liver is a target site for estrogens, we have characterized hepatic receptors in cytosol and nucleus. Both male and female hepatic cytosol receptors have two components; K_d 2×10^{-10} and 5×10^{-9} . Male and female receptors are also similar in quantity and substrate specificity. Hepatic nuclear receptors have two conformations exhibiting sedimentation coefficients of 4S and 5S.

Nuclear translocation was accomplished in a cell free system and was temperature dependent. Parallel experiments with uterine cytosolic estrogen-receptor complexes resulted in translocation of 5S receptor only. Experiments are in progress to assess the role of hepatic estrogen receptors in the modulation of hepatic enzymes by endogenous steroids as well as hormonally-active xenobiotics. Emphasis will be placed on age-dependent estrogen action in the liver.

Sex-dependent differences in the repression of hepatic mixed-function oxidases (mfo) after *in vivo* Cd treatment have been characterized; male mfo are repressed whereas female enzymes are not. This sex difference is pituitary-dependent and is only manifested in postpubertal animals. Following treatment of newborn rat (2,4,6) with DES, the resulting adult male and female liver enzymes were unaffected by Cd *in vivo*. These data indicate permanent feminization in the response of mfo systems to Cd following newborn exposure to DES. Preliminary experiments, in which rats received vehicle, DES or testosterone on days 2,4, and 6 and Cd on day 56, revealed that newborn exposure to DES protects against Cd castration in adult animals. Future experiments will attempt to elucidate the mechanism of DES protection emphasizing hormonal factors and sensitive indicators of testicular function.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The pharmacodynamic properties of environmental agents in perinatal systems can be an extremely important determinant of target organ sensitivity to various forms of developmental toxicity. Susceptibility to classes of chemicals during the period of development and differentiation is often determined by metabolism (toxication or intoxication), transport, and binding of the parent compound and toxicologically-important metabolites. Placental transfer rates of chemicals as well as the partition into milk are of particular interest for perinatal pharmacology studies. We have concentrated our efforts on organochlorine agents that cause or have the potential to cause serious environmental health hazards such as the PCBs, PBBs, TCDD, heavy metals such as cadmium and mercury, and synthetic steroids such as diethylstilbestrol. The perinatal toxicology of hormonally-active xenobiotics is emphasized due to the obvious importance of hormonal control to differentiation and development. The experiments are designed so that structure-activity relationships are elucidated for the perinatal toxicology of groups of chemicals. The perinatal pharmacology studies provide direction for subsequent target-organ toxicity studies. For example, the unique pharmacological properties of 4-CB prompted us to evaluate the developmental toxicity of this isomer which led to the finding that 4-CB is a potent behavioral teratogen. The organohalogenes, like many xenobiotics, possess direct or indirect hormonal activity. Therefore we are evaluating the role of hormone receptors in developmental toxicity including nuclear translocation. Moreover, the role of estrogen receptors in the modulation of hepatic enzymes by hormonally-active xenobiotics is being investigated. Also of interest is the ability of xenobiotics to alter neonatal programming of hepatic metabolism and the role that such alterations might have in developmental toxicity. One interesting example is our finding that neonatal feminization

by DES protects against testicular toxicity of Cd in subsequent adult animals whereas neonatal androgen exposure has the opposite effect.

PUBLICATIONS

Lucier, G. W., Sonawane, B. R., and McDaniel, O. S.: Glucuronidation and deglucuronidation reactions in hepatic and extrahepatic tissues during perinatal development. Drug Metab. Distribution. 5: 279-287, 1977.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70131-02 LET
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less)		
Development of Intestinal Functions: Normal and Toxic Responses		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: OTHER:	C. M. Schiller R. J. Shirkey G. W. Lucier B. A. Fowler J. S. Woods E. E. McConnell	Senior Staff Fellow Visiting Fellow Head, Developmental Toxicology Research Biologist Head, Biochemical Toxicology Veterinary Pathologist LET LET LET LET LET EBCB
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COOPERATING UNITS (if any) Environmental Biology and Chemistry Branch, NIEHS Research Triangle Institute, RTP		
LAB/BRANCH Laboratory of Environmental Toxicology		
SECTION Developmental Toxicology Group		
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TOTAL MANYEARS: 4.6	PROFESSIONAL: 4.1	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline key words) It is the long-range purpose of this project to study the <u>perinatal development</u> of the functioning <u>small intestine</u> in normal and <u>environmentally-, nutritionally- and hormonally-stressed</u> animals. The topics of present interest which relate to the development of the normal functioning small intestine are: (1) ontogeny of <u>lactate dehydrogenase isozymes</u> , <u>kinetic parameters</u> and <u>substrate/cofactor specificity</u> during intestinal development, (2) characterization of the <u>morphology</u> and <u>metabolism</u> of isolated intestinal absorptive <u>tip cells</u> , (3) determination of the <u>isozyme profile</u> for <u>β-glucuronidase</u> during intestinal development and after <u>1,2-dimethylhydrazine treatment</u> , (4) differential susceptibility of <u>isolated intestinal tip and crypt cells</u> to <u>2,3,7,8-tetrachlorodibenzo-p-dioxin induction</u> , (5) mechanism of <u>As-induced alterations of mitochondrial function</u> in normal and <u>alloxan-diabetic</u> animals, and (6) monitoring the <u>response</u> of key intestinal enzymes to <u>in vivo steroid treatments</u> .		

PROJECT DESCRIPTION

METHODS EMPLOYED: The preparation of the different subcellular fractions from the various intestinal tissues as well as the numerous enzyme assays have been described in detail before and are contained in the bibliographic references. Of special interest are the methods we adapted or devised for the lactate dehydrogenase, isolated intestinal cell and As-diabetes studies.

Lactate dehydrogenase was monitored throughout development by measuring both the forward and reverse reactions with lactate and pyruvate as substrates respectively, as well as with high and low concentrations of pyruvate in the presence of NADH or NAD⁺. The activities of intestinal lactate dehydrogenase in the presence of NADH-high pyruvate and NAD⁺-low pyruvate were correlated with the expected isozyme profile. At each developmental stage the isozyme profile was determined by electrophoresis on prepared agarose gels with polyester film supports in tris-citrate-barbital buffer. The bands of enzyme activity were visualized with TNBT, PMS and NAD with lactate as the substrate. The ontogeny of the kinetic parameters K_m and V_{max} was determined for both the forward and the reverse reactions. The linear regression coefficients were determined by the method of least squares by the Lineweaver-Burke and Eadie-Hofstee plots. The values obtained for intestinal tissue were compared to those obtained from both heart and muscle in these same animals. The isolated intestinal tip and crypt cells utilized for the time-course of TCDD induction of UDP-glucuronyltransferase were prepared by the method of Harrison and Webster. This is a low-amplitude, high-frequency vibration technique which is used before and after insufflation of the everted intestinal segments. For our detailed morphologic and metabolic studies of the isolated intestinal tip cells, the vibration method of preparation was compared to an enzyme method of preparation. Several sources of proteases were examined under a wide-range of experimental conditions. The cells from both methods of preparation were examined under light, phase contrast, transmission electron and scanning electron microscopes. Of particular interest to us were the capacities of these cells to utilize glucose and various other substrates as energy sources.

The As studies involved animals that were exposed to oral arsenic (0 or 40 ppm sodium arsenate [As^{+5}]) for three weeks in the drinking water. After three weeks, half of each dose group were injected (i.v.) with alloxan-monohydrate (50 mg/kg). These animals were used for *in vitro* studies in which the rate of hepatic lipogenesis was measured with ^{14}C -glucose, ^{14}C -pyruvate on ^3H - H_2O ; the alteration to urinary enzyme levels was measured and the morphology of the various tissues was examined.

MAJOR FINDINGS AND PROPOSED COURSE: The observed developmental pattern of lactate dehydrogenase indicated a marked increase in the early postnatal activity of the reverse reaction. The peak in the activity of the forward reaction was not as marked. Evaluation of the isozymic profile with developmental age revealed that both fetal and adult intestinal lactate dehydrogenase

is predominantly M_4 based on migration compared to a mammalian standard which contains purified isozymes and lactate dehydrogenase samples of fresh heart and muscle tissue prepared from the same developing animals. During the post-natal period there is a gradual increase in the predominantly heart-type (H_4) isozyme and disappearance of the muscle-type (M_4) followed by the gradual reappearance of the M_4 and the disappearance of the H_4 . This developmental intestinal lactate dehydrogenase profile was correlated with the observed $NH_4^+DH_L/NADH_H^+$.

We observed that isolated intestinal tip cells can be prepared by the enzymic method under conditions such that a uniformly monodisperse cell suspension results. These cell suspensions repeatedly excluded trypan blue dye at a level of 99%. However, close examination of these cells under several microscopes revealed that these cells had lost their columnar shape and contained unusually larger numbers of vacuoles and a nucleus which was located in the more basal regions. Metabolic studies initially indicated poor rates of oxygen consumption and response to added substrates in the cells prepared by the enzymic method. The isolated intestinal cells prepared by vibration were observed to be in clumps which made trypan dye exclusion and cell counts difficult. Comparisons were made on a protein basis. These cells were shown to utilize glucose in an incubation medium linearly with time for at least an hour. Also, the response of these cells to endogenous substrates and inhibitors was monitored with the oxygen electrode.

Total intestinal β -glucuronidase activity reaches a developmental peak in Fischer rats during the second week after birth. Our studies include the comparison of intestinal β -glucuronidase isozymes at several stages of development with those of adult liver tissue, of intestinal tissue from DMH-treated rats, and of colonic tumors from DMH-treated rats. Initial data indicate that intestinal β -glucuronidase is predominantly cytosolic as compared to that of liver which is predominantly membrane bound lysosomal and microsomal. Partial purification of β -glucuronidase revealed two peaks of β -glucuronidase activity from adult intestinal tissue and only one peak, with a distinctive R_f value, from intestine of DMH-treated rats sacrificed 33 weeks after treatment.

The cell specific induction of uridine diphosphate (UDP)-glucuronyltransferase by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in intestinal epithelium was studied by administering ^{14}C -TCDD (8 $\mu g/kg$) to adult female rats. Intact epithelial cells from the tip and crypt regions were isolated by differential vibration of rat duodenum. Cell separation was monitored by electron microscopy and marker enzymes. UDP-glucuronyltransferase and radioactivity were assayed in both cell types 0 hr, 3 hr, 10 hr, 1 day, 3 days and 5 days after treatment. UDP-glucuronyltransferase activities were not significantly changed in either cell type isolated from TCDD-treated rats until 24 hrs after treatment when a three-fold increase in crypt cell activity was evident. No significant changes in UDP-glucuronyltransferase activity were observed in the differentiated tip cells until three days after TCDD treatment. UDP-glucuronyl

transferase was increased approximately two-fold in both cell types from three and five days following TCDD treatment. There was a negative correlation between the time-course of UDP-glucuronyltransferase induction and the ^{14}C -TCDD concentrations measured in these cells. These studies suggest that the undifferentiated cells of the intestinal crypt region are more sensitive to TCDD inductive actions than are the absorptive tip cells.

Oral arsenic exposure results in alterations to mitochondrial function in several tissues. Also arsenic accumulates in the kidney causing swelling of the microtubules. Earlier studies from our laboratory revealed that arsenic specifically interfered with the normal rates of mitochondrial pyruvate utilization. Male rats were given access to deionized drinking water containing 0, 40 or 80 parts per million (ppm) sodium arsenate (As^{+5}) for three weeks. Respiration studies with mitochondria isolated from treated animals indicated decreased state 3 respiration (with ADP) and decreased respiratory control ratios (RCR) for pyruvate/malate-mediated respiration, but not for succinate-mediated respiration, as compared to control respiration values. In addition, pyruvate dehydrogenase activity was measured, in both liver and intestine, before and after Mg-activation *in vitro*. After three weeks, the effects of arsenic at the highest dose level were pronounced on the basal pyruvate dehydrogenase activity (before activation) as well as the total pyruvate dehydrogenase (after activation). The inhibition of pyruvate dehydrogenase activity both before and after Mg-activation suggests an arsenic effect on mitochondrial pyruvate metabolism which, in part, involves inhibition of pyruvate decarboxylase. Evidence is also presented which may indicate an arsenic effect on the kinase and/or phosphatase which regulate pyruvate dehydrogenase activity.

Currently, we evaluated the possible exacerbation of arsenic exposure by diabetes. Rates of hepatic lipogenesis were measured in 0 and 40 ppm arsenic-treated normal and alloxan-diabetic rats with $\text{U-}^{14}\text{C}$ -glucose, $2\text{-}^{14}\text{C}$ -sodium pyruvate and $^3\text{H-H}_2\text{O}$. If arsenic interferes with mitochondrial pyruvate utilization, then a difference in the rate of lipogenesis should be observed with both glucose and pyruvate, but not with $^3\text{H-H}_2\text{O}$. Our initial experiments indicate such an effect which is exacerbated by alloxan-diabetes. However, the experimental variation is too high to make any definitive conclusions.

In contrast, the results from the urinary enzyme measurements from these same animals support several statistically significant ($2p < 0.10$) observations: (1) lactate dehydrogenase, L-glutamate oxaloacetate transaminase, acid phosphatase, β -glucuronidase and N-acetyl- β -D-glucosaminidase are elevated in the alloxan-diabetic arsenic rats as compared to either controls of the normal arsenic rats; (2) alkaline phosphatase is elevated in the normal arsenic rats as compared to the normal controls; and (3) acid phosphatase is elevated in the alloxan-diabetic control rats as compared to normal control rats.

The results from the hormonal responsiveness pilot experiments indicated that the flavoprotein-glycerol 3-phosphate dehydrogenase (a mitochondrial marker enzyme and a key enzyme in mitochondrial energy metabolism) is responsive to alterations to the pituitary and to testosterone after castration, but not to testosterone normally or after castration and hypophysectomization.

We plan to concentrate our efforts in fiscal year 1979 on follow-up studies based on several of our current projects. In particular, we are interested in studying (1) the effects of developmental lactate dehydrogenase isozyme patterns on the lactate and pyruvate output of this tissue, (2) utilization of the isolated intestinal tip cells for evaluating additional aspects of (a) energy metabolism, (b) effects of TCDD on cell metabolism, and (c) morphologic development (3) further comparisons of the β -glucuronidase isozyme profile with rat colonic tumors from DMH-treated rats and neonatal intestinal tissue as well as using histochemistry to verify the intracellular β -glucuronidase activity in hepatic and intestinal tissues (4) effects of non-steroidal hormones on Fp-glycerol 3-phosphate dehydrogenase programmed response in normal and surgically-treated animals (5) role of glutamine metabolism during development in intestinal energy metabolism and (6) continuation of mechanistic studies which further clarify sites of subcellular effects.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In the past, perinatal development studies of the small intestine have dealt primarily with hydrolytic enzymes, the brush border, and the absorption of specific nutrients. Our emphasis has been on the development of the metabolic energy producing processes required for the active transport of nutrients and also the effect of various bioactive environmental compounds on the normal developmental patterns of this target organ. Currently, our laboratory is using a variety of biochemical and morphological techniques to study several aspects of the development of normal intestinal function, mechanisms of intestinal toxicity and indicators for response to toxic insults.

Many of our results have long-range significance in providing a better understanding of the development of normal intestinal function, the normal morphology and metabolism of intestinal cells and the unique mitochondrial and energy metabolism of intestinal tissue. Other observations include results which may have toxicological and clinical utility for the medical community. Examples of the latter include our studies on the responses to treatments with 1,2-dimethylhydrazine, 2,3,7,8-tetrachlorodibenzo-p-dioxin and arsenic.

PUBLICATIONS

Fowler, B. A., Woods, J. S., and Schiller, C. M.: The ultrastructural and biochemical effects of prolonged oral arsenic exposure on liver mitochondria of rats. Environ. Health Perspec. 19: 197-204, 1977.

Schiller, C. M., Fowler, B. A., and Woods, J. S.: Arsenic effects on pyruvate dehydrogenase activation. Environ. Health Perspec. 19: 205-207, 1977.

Schiller, C. M.: Pyruvate dehydrogenase activity in hamster small intestine during development. Biochem. J. 164: 693-697, 1977.

Schiller, C. M., and Walden, R.: Development of substrate cycle enzymes in the hamster small intestine. Develop. Biol. 60: 130-138, 1977.

Schiller, C. M. and Woods, J. S.: Effects of heavy metals on organ systems: Gastrointestinal tract and liver. (conference proceedings).

Schiller, C. M. and Lucier, G. W.: The differential response of isolated intestinal crypt and tip cells to the inductive actions of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Chem. Biol. Interactions (in press).

Schiller, C. M., Fowler, B. A., and Woods, J. S.: Pyruvate metabolism after in vivo exposure to oral arsenic. Chem. Biol. Interactions (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70135-02 LET						
PERIOD COVERED October 1, 1977 to September 30, 1978								
TITLE OF PROJECT (80 characters or less) The Effect of Hormones and Xenobiotics on the Regulation of Perinatal Enzyme Development								
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT								
PI: OTHER:	C. A. Lamartiniere G. W. Lucier C. S. Dieringer W. Powell-Jones N. Illsley E. Kita E. Lui	Senior Staff Fellow Head, Developmental Tox. Staff Fellow Visiting Fellow Visiting Fellow Guest Worker Visiting Fellow COOPERATING UNITS (if any) None LAB/BRANCH Laboratory of Environmental Toxicology SECTION Developmental Toxicology Group INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709 <table style="width: 100%; border: none;"> <tr> <td style="border: none;">TOTAL MANYEARS:</td> <td style="border: none;">PROFESSIONAL:</td> <td style="border: none;">OTHER:</td> </tr> <tr> <td style="border: none; text-align: center;">2.5</td> <td style="border: none; text-align: center;">1.0</td> <td style="border: none; text-align: center;">1.5</td> </tr> </table>	TOTAL MANYEARS:	PROFESSIONAL:	OTHER:	2.5	1.0	1.5
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:						
2.5	1.0	1.5						
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS								
SUMMARY OF WORK (200 words or less - underline keywords) Our research efforts are directed toward investigating the effect and mode of action of <u>hormones and xenobiotics on the regulation of enzyme patterns during perinatal development</u> . Particular emphasis has been placed on the postnatal development of hepatic enzymes in the rat following exposure to <u>testosterone propionate (TP)</u> , <u>diethylstilbestrol (DES)</u> , and other hormonally active <u>xenobiotics (polychlorinated biphenyls [PCB])</u> . These enzyme markers are characterized by low catalytic activity during normal prepubertal life but increasing values in the adult rat resulting in enzyme levels which are significantly higher in one sex than the other. We report here the postnatal <u>programming</u> of five sex-dependent hepatic enzyme markers by TP and/or DES and the results of our studies on the role of the <u>hypothalamic-pituitary-gonadal axis</u> on regulation of some of these enzymes. We also report our findings on the effect of PCB gestational exposure on enzyme markers in these offsprings.								

PROJECT DESCRIPTION

METHODS EMPLOYED: Postnatal programming was carried out using Sprague Dawley CD rats that were cross-fostered on day 1 following parturition. On days 2, 4 and 6 they were injected with either 0.02 ml propylene glycol (PG) or 1.45 μ moles TP or DES. The PCBs were administered orally using corn oil as a vehicle on alternate days from 8-18th day of gestation. Transplantation of pituitaries were done by placing pituitaries of age-matched rats under the kidney capsule of hypophysectomized rats anesthetized with nembutal.

MAJOR FINDINGS AND PROPOSED COURSE: Visual observations of postnatally programmed rats revealed that testes of normal males were descended at 21 days but those of TP and DES animals descended later (35 and 63 days respectively). These TP and DES treated animals had smaller testes at prepubertal and adult ages. Vaginal openings in normal females occurred around 42 days of age but vaginal openings in TP and DES treated animals did not occur through 63 days of age. While control and TP females had similar uterine wet weights at 21 days of age, DES females had heavier uteri (may be due to higher circulating estrogens from DES treatment). In contrast adult DES and TP females had uterine weights lower than that of controls. An attempt to produce a uterotrophic response in 21 day old females was positive in control and TP females, but no elevation in uterine wet weights occurred in DES females. However, exogenous E_2 administration to adult females, did elicit a uterotrophic response in control and TP and DES animals.

The various enzyme markers that we have investigated as indicators of postnatal programming are histidase, 5- α reductase, UDP glucuronyltransferase, 16- α hydroxylase, ethylmorphine demethylase, aminopyrine demethylase, and glutathione-S-transferase. Early postnatal administration of TP and DES had no significant effect on these enzymes at 21 days of age but alterations were observed in some of these enzyme markers at 63 days of age. Histidase activity, which is normally higher in females than males, was masculinized (decreased) by DES in adult females. Adult male glucuronyltransferase, 16 α -hydroxylase, ethylmorphine demethylase, and aminopyrine demethylase (activities of these enzymes are normally higher in adult males than females) were feminized by neonatal TP and DES. Since no effect is seen in young animals and effects are seen in adult animals, TP and DES appear to be capable of eliciting a programming effect by feminizing hepatic UDPGT, 16 α hydroxylase, ethylmorphine demethylase and aminopyrine demethylase in adult males. DES treatment of newborns masculinized histidase in the adult female. 5- α reductase and Glutathione-S-transferase activities were unchanged at all ages tested.

In an attempt to understand the mechanism of postnatal programming of hepatic enzymes, we have investigated the role of the hypothalamic-pituitary-gonadal axis. Gonadectomy of normal rats revealed that high female histidase levels are dependent on the ovaries and that estrogen is responsible for these higher levels in the adult females. Hypophysectomy of males results in a dramatic

increase in activity. However upon transplantation of a pituitary under the kidney capsule of hypophysectomized male or female rats no significant effect was noted. Likewise subcutaneous injections of FSH and LH to hypophysectomized rats had no effect. Castration of male or female rats had no significant effect on adult glutathione-S-transferase enzyme activities but hypophysectomy resulted in significantly elevated enzyme levels in both sexes. Pituitary transplants in these hypophysectomized animals decreased glutathione-S-transferase activity to levels intermediate to those of hypophysectomized and normal animals. Subcutaneous injections of FSH and LH to hypophysectomized male and female rats had a similar effect as the ectopic pituitary. These experiments suggest the involvement of the hypothalamic-pituitary-gonadal axis in the regulation of cytosolic histidase and glutathione-S-transferase.

In our 1977 annual report we demonstrated that gestational exposure to DES results in prenatal programming of hepatic histidase in the rat. These studies have been extended to gestational exposure of rats to PCBs. Our experiments demonstrate that 3,3',4,4'-tetrachlorobiphenyl (4-CB) resulted in lower histidase levels in 6 day old offsprings. Activity levels were, however, back to normal in 20 day old animals. While glutathione-S-transferase was unaffected by the 4-CB, positive modulation was observed in 6 to 55 day old offspring of 2,2',4,4',5,5'-hexachlorobiphenyl (6-CB) exposed (gestational) rats. These results indicate that hormonally active xenobiotics administered during gestation are capable of altering the normal sequence of postnatal development.

We are currently expanding these investigations to the measurement of circulating gonadotrophins, pituitary hormones and specific receptors and correlating behavioral changes between control and treated animals. We are also evaluating hepatic protein profiles of normal and programmed animals by two-dimensional gel electrophoresis. Since a pituitary requirement is necessary for complete and normal expression of the developmental pattern of these hepatic enzymes, we are initiating in vitro studies to complement our present ongoing in vivo investigations. Using an established hepatoma cell line, we are studying the effect of hormones, pituitary extracts and hormonally active xenobiotics on metabolism. This in vitro model will enable us to better elucidate the role of the pituitary in the regulation of hepatic metabolism and the mechanism whereby chemicals can alter this process.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: One of the dominant biochemical aspects of the orderly development of an organism from fertilization to maturity is the continuous progression of enzyme protein formation ultimately resulting in specific enzymatic patterns which constitute and determine the metabolic machinery characteristic of each mature differentiated tissue. The postnatal development of sex-dependent hepatic mammalian enzymes is an excellent system to investigate ontogeny. It is the purpose of our studies to further our understanding of the factors responsible for complex postnatal developmental patterns. In addition to studying the normal expression of these enzymes we want to see if xenobiotics can alter the normal expression

and discover, if so, how this is accomplished. It is quite obvious that in our complex society and environment the fetus and newborn are exposed to numerous factors that can have deleterious effects on the developing offspring. The studies reported above are examples of alterations of enzyme expression that may be markers for abnormal differentiation and development. These effects on enzyme development may result in an altered capacity to metabolize potential teratogens, carcinogens, or drugs and, therefore, allow humans to be more susceptible to biochemical insult. An understanding of these biochemical mechanisms should allow us to make predictions of chemicals that might alter the normal sequence of programming.

PUBLICATIONS

Lamartiniere, C. A. and Feigelson, M.: Effects of estrogen, glucocorticoid, glucagon and adenosine 3': 5' monophosphate on the catalytic activity, amount and rate of de novo synthesis of hepatic histidase. J. Biol. Chem. 252: 3234-3239, 1977.

Lamartiniere, C. A. and Lucier, G. W.: Programming of hepatic histidase following prenatal administration of diethylstilbestrol. J. Steroid Biochem. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70138-02 LET
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) <u>Effects of Environmental Factors on Programming of Steroid Metabolizing Enzymes</u>		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: OTHER:	C. S. Dieringer G. W. Lucier C. A. Lamartiniere	Staff Fellow Head, Developmental Tox. Senior Staff Fellow
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		NIEHS NIEHS NIEHS
COOPERATING UNITS (if any) None		
LAB/BRANCH <u>Laboratory of Environmental Toxicology</u>		
SECTION <u>Developmental Toxicology Group</u>		
INSTITUTE AND LOCATION <u>NIEHS, NIH, Research Triangle Park, North Carolina 27709</u>		
TOTAL MANYEARS: 1.3	PROFESSIONAL: 1.0	OTHER: 0.3
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline !) This work examines the phenomenon of <u>programming (imprinting) of steroid metabolizing enzymes</u> in the rat in an effort to determine the role of the endocrine system in this process. Initial studies examine the dual localization of 5α-reductase in the microsomes and nuclei of the immature and mature rat, both male and female. The effects of early <u>neonatal exposure to testosterone propionate or DES</u> on normal programming of adult enzyme activities or on <u>enzyme responsiveness to hormonal stimulation</u> are examined. The ability of pure <u>PCB isomers</u> to alter normal programming of hepatic steroid metabolizing enzymes when administered prenatally is also investigated. Future studies will attempt to further elucidate the role of the <u>hypothalamo-pituitary-gonadal axis</u> in the regulation and modulation of hepatic 5α-reductase and 16α-hydroxylase activities.		

PROJECT DESCRIPTION

METHODS EMPLOYED: These studies examined the effects of hormonally-active environmental agents on steroid metabolizing enzyme activity. Specific chemicals included in the studies are DES, selected PCB isomers and certain steroid hormones. Environmental agents were administered prenatally (p.o. administration to pregnant rats on selected days of gestation) or postnatally (s.c. administration to newborn rats during the first week of life). Some animals were subjected to various surgical procedures (i.e. hypophysectomy and/or castration) prior to being treated with testosterone propionate, DES or dihydrotestosterone (days 56-63, s.c.). Following treatment, enzyme activities were studied in the immature (20 days old) or mature (63 days old) rat, to determine the effect of the various treatments on enzyme programming.

5 α -reductase (5 α -RED) and 16 α -hydroxylase (16 α -OH) activities were determined in microsomal fractions of the rat liver. Enzyme activities were determined using radioactive substrates; subsequent radioactive metabolites were separated by thin layer chromatography and quantitated by liquid scintillation counting.

Subcellular tissue fractions were obtained using standard procedures. Purified liver nuclear preparations were prepared for measurement of 5 α -RED activity by the method of Gustafsson and Pousette (Biochem. 13: 875, 1974), using discontinuous sucrose gradients.

MAJOR FINDINGS AND PROPOSED COURSE: "Programming" is defined as the ability of hormonally-active chemicals administered during a critical period of early development (i.e. just before or just after birth) to predetermine the subsequent level of enzyme activities in the resulting adult animal. These effects are commonly referred to as masculinizing or feminizing effects; therefore, altered programming is more evident in enzymes which normally exhibit marked differences in activity between male and female animals. For these reasons we selected as our enzyme indicators the two enzymes which we previously demonstrated to exhibit sexual differentiation in the adult, e.g. 16 α -hydroxylase (M>F) and 5 α -reductase (F>M). Both 16 α -OH and 5 α -RED are located in the endoplasmic reticulum, but 5 α -RED is also found in the nucleus. These studies have shown that the specific activity of hepatic 5 α -RED is about 5-fold higher in the microsomal fraction than in the nuclear fraction, regardless of the sex or the age of the rat (21 day immature vs. 63 day mature).

Neonatal treatment (days 2, 4 and 6) with testosterone propionate (TP) or diethylstilbestrol (DES) had no significant effect on the normal development of hepatic microsomal 5 α -RED activity in the male or female rat, at either 22 (immature) or 63 days of age (mature). In contrast 16 α -OH activity was altered in the adult animal by neonatal treatment with TP or DES. DES treatment feminized (decreased) 16 α -OH in both the intact and castrate (day 1) male rats by 63 days of age. TP also feminized adult 16 α -OH activity when administered neonatally to the intact male rat. Adult hepatic enzyme activity in the female

rat was unaltered by neonatal exposure to either compound. These studies demonstrate that the programming of 16α -OH can be altered by neonatal exposure to hormonally active compounds, and that 5α -RED and 16α -OH are under different control systems.

In addition to being "programmable" by neonatal exposure to testosterone, some enzymes are also responsive to postpubertal exposure to sex hormones. In an effort to more fully elucidate the mechanism by which 5α -RED and 16α -OH are regulated, these studies have examined the effects of castration (CASTR) on day 42, hypophysectomy (HYPOX) on day 21, or CASTR plus HYPOX on adult (day 65) hepatic enzyme activity. The degree of responsiveness to 17β -estradiol (E_2) or TP treatment (100 μ g/kg s.c. on days 58-64) was also determined. Both enzymes were feminized following castration of the M rat; i.e. 5α -RED was increased and 16α -OH was decreased. In contrast, removal of the ovaries did not alter F enzyme activity. 5α -RED was virtually abolished in both sexes after HYPOX or HYPOX-CASTR. 16α -OH was significantly decreased in M rats following HYPOX, and was diminished by the same degree after HYPOX-CASTR. Conversely, in the F rat 16α -OH was significantly increased following CASTR, and was increased by the same degree after HYPOX-CASTR. Female enzyme activities were unresponsive to either E_2 or TP treatment regardless of the surgical status of the animal. However, in the intact M rat, E_2 feminized both 16α -OH (decreased) and 5α -RED (increased), whereas TP had no significant effect on intact M enzyme activities. In the CASTR M rat, TP administration resulted in a masculinization of both enzymes; i.e. 16α -OH was increased, and 5α -RED was decreased. There was no response to E_2 in the CASTR M rat. Enzyme levels in HYPOX or HYPOX-CASTR M rats were unaltered by either E_2 or TP. These studies indicate that hepatic 16α -OH and 5α -RED are significantly modulated by E_2 and TP in the M rat, whereas these steroids have no effect on the corresponding steroid-metabolizing enzymes in the F rat. The enzyme responsiveness to hormone administration in male rats requires an intact pituitary and might be programmed by neonatal exposures to androgens.

Pure PCB isomers were examined for their ability to alter the normal development of rat hepatic 5α -RED and 16α -OH activities. Isomers examined were 4-chlorobiphenyl (1-CB), 3,4-3'4'-tetrachlorobiphenyl (4-CB) and 2,4,5-2',4',5'-hexachlorobiphenyl (6-CB). Prenatal exposure to 1-CB had little or no effect on either 5α -RED or 16α -OH at -1, 6, 20 or 55 days of age. Similarly, 4-CB did not alter 16α -OH activity at any age. On the other hand, 4-CB produced a prepubertal (-1, 6 and 20 days) repression of 5α -RED, while adult (55 days) 5α -RED activity in either sex was unaltered. Prenatal exposure to 6-CB had little effect on 5α -RED activity, but had profound inductive effects on 16α -RED at 6 and 20 days of age in the male and female, and remained high in both sexes at 55 days of age (masculinization).

These studies show that 5α -RED and 16α -OH exhibit markedly differing response to the PCBs, and that the degree and/or position of biphenyl chlorination affects PCB-induced alterations of normal enzyme development.

Future studies will attempt to further elucidate the mechanism by which programming of steroid metabolizing enzymes occurs. The role of the endocrine system in this process will be further investigated through the continued use of various animal surgical procedures. For example, examination of enzyme activities in castrated, hypophysectomized, or castrated-hypophysectomized animals, will help us to determine the role of the hypothalamo-pituitary-gonadal axis, and to determine which element of this axis is the most important. The use of pituitary transplants into surgically manipulated animals will further enhance our understanding of how this axis regulates enzyme activities. A comparison of the effects of xenobiotics on various enzyme systems could enable us to determine whether more than one factor is operating in the programming process. In addition, use of an HTC tissue culture system will enable us to determine the role of various factors, including pituitary extract, LH, FSH or prolactin, in the regulation of enzyme activity. A more thorough characterization of the factors which are responsible for sexual differentiation of hepatic steroid metabolizing enzyme activity, and which of these factors are altered by exposure to xenobiotics during the critical period of development, is necessary in order to determine which parameters should be measured as predictive indicators of developmental toxicology.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Recent reports in the literature have demonstrated that neonatal testosterone administration is capable of altering adult hepatic steroid metabolizing enzyme activities. It is also known that the administration of various hormones during the first week of life can interfere with the normal sexual differentiation of the rat brain. With these facts in mind, it becomes important to recognize the possible consequences which may result following exposure to a hormonally-active environmental agent during a critical phase of development. Such an exposure could alter the normal processes which program steroid metabolizing enzyme systems or sexual differentiation of the brain, with permanent effects on the exposed animal. These studies are designed to characterize the role the endocrine system plays in the normal programming of hepatic steroid metabolizing enzymes in the rat. They further attempt to determine how xenobiotics affect normal neonatal hepatic or testicular enzyme programming mechanisms, to define what role such alterations play in developmental toxicity, and to determine whether such a system can be used as a model for predicting developmental toxicity.

PROJECT DESCRIPTION

Various approaches are utilized to assess the functional relationship between heme biosynthetic capability and hemoprotein synthesis and activity in mammalian tissues. These include (1) assessment of the time and dose-related effects of agents known to specifically inhibit or otherwise alter heme biosynthesis and degradation on the synthesis and functional activation of mitochondrial and microsomal cytochromes in mammalian tissues in vivo; (2) studies of the effects of heme on the turnover of tissue cytochromes following specific inhibition of ribosomal or mitochondrial protein synthesis in vivo; and (3) cytochrome recombination studies in vitro performed to assess the role of heme in the synthesis and functional activation of holocytochromes from induced apoproteins following acute and chronic impairment of heme biosynthesis in various tissues. Rates of protein and heme biosynthesis are assessed using pulse-labeling techniques with appropriate radioisotopes. In the assessment of chronic toxicity, biochemical and morphological studies are performed to determine the effects of prenatal or prolonged exposure to various hematotoxic agents on the growth and function of tissue organelles and hemoproteins. Development of tests for predicting hematotoxicity in animals as well as human populations are based on identification of early changes in specific patterns of excreted or circulating heme precursors associated with prolonged exposure to selected environmental agents. Porphyrins and heme precursors are measured by spectrophotometric and fluorometric techniques.

MAJOR FINDINGS AND PROPOSED COURSE: Results of recent studies indicate that the regulation of heme biosynthesis and metabolism in mammalian tissues may be exquisitely sensitive to alteration by environmental metals and their salts. Since non-transition metals such as indium and thallium are as effective in this regard as iron, cobalt and other metallic elements which are capable of undergoing reversible changes in oxidation state, it is suggested that this action is mediated via a direct effect of the metal at the level of synthesis of regulatory and other enzymes in the heme biosynthetic and degradative processes, and that formation of a metalloporphyrin is therefore not required for regulatory significance. Chronic exposure to low levels of trace metals such as arsenic and mercury also results in specific alterations in heme biosynthetic and degradative enzymes in various tissues over a prolonged time course of exposure. These effects are manifested by changes in excreted and circulating levels of heme precursors which are specific for the metal involved. Results of these studies are being assessed for their utility in the design of predictive tests for determining sub-toxic exposure to specific trace metals in occupationally exposed humans.

Studies with 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) have shown that this substance, although extremely toxic in some organ systems, does not induce hepatic heme biosynthesis in mammals. This observation suggests that TCDD-related prophyria may be incurred via impairment of porphyrin metabolism or

some other process which is not directly related to heme biosynthesis in mammals. In contrast, TCDD does induce the synthesis of a specific form of the cytochrome P-450, P-448, and studies now in press (Kitchin and Woods, Molec. Pharmacol, 1978) suggest that the mechanism of TCDD induction of P-448 synthesis involves de novo induction of the apocytochrome portion of the molecule, which then combines in vitro with added heme to form the functional cytochrome. Presumably, TCDD induces cytochrome P-448 synthesis in vivo through this mechanism.

Other studies associated with the regulation of heme biosynthesis in mammalian tissues are oriented toward development of reliable assay procedures for measuring the activities of uroporphyrinogen decarboxylase and coproporphyrinogen oxidase, two enzymes of the heme biosynthetic pathway which have heretofore been difficult to assay because of the instability of their porphyrinogen substrates and products. Recent progress has been made toward overcoming these problems, however, and it is anticipated that the role of these enzymes in the regulation of heme biosynthesis and in the development of porphyrinogenic responsiveness to metals and other hematotoxic environmental agents will be determined within the foreseeable future.

Studies concerning the role of heme in the regulation and functional activation of hemoproteins in mammalian tissues will continue. Particular emphasis will be placed on understanding factors affecting the synthesis and regulation of mitochondrial and microsomal cytochromes in mammalian tissues and the effects of chronic exposure to agents which impair heme biosynthetic capability on these processes. Specific studies will be focused on investigation of the mechanisms by which various trace metals selectively interact with heme biosynthetic and metabolic processes, and on further elucidation of the mechanism of TCDD induction on mixed function oxidase activity in mammalian liver. In addition, findings from these studies will be utilized in the design of tests for assessing the potentially toxic effects to humans who are chronically exposed to hematotoxic agents in the environment.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Knowledge of the mechanisms by which environmental agents selectively exert toxic effects on cellular growth and function is essential to the development of a biochemical basis for both understanding and preventing chronic organ toxicity. The proposed studies are designed to develop such knowledge in the area of heme biosynthetic function through investigation of the mechanisms of regulation of heme metabolic and degradative processes in mammalian tissues, and through elucidation of the effects of prolonged exposure to hematotoxic agents on the biosynthesis and function of specific regulatory hemoproteins. It is anticipated that these studies will contribute to an enhanced understanding of the problems and mechanisms of chronic organ toxicity resulting from impaired heme biosynthetic capability and hemoprotein function, and will promote the more efficacious identification of potentially harmful hematotoxic environmental substances. In addition, these studies will enhance the design of clinically

useful tests for assessing subtoxic exposure to specific environmental agents in human populations.

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Fowler, B. A. and Woods, J. S.: Ultrastructural and biochemical changes in renal mitochondria following chronic methyl mercury exposure: The relationship to renal function. Exp. Molec. Pathol. 27: 403-412, 1977.

Woods, J. S. and Fowler, B. A.: Renal porphyrinuria during chronic methyl mercury exposure. J. Lab. Clin. Med. 90: 266-273, 1977.

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Schiller, C. M., Fowler, B. A., and Woods, J. S.: Arsenic effects on pyruvate dehydrogenase activation. Environ. Health Perspec. 19: 204-208, 1977.

Woods, J. S. and Fowler, B. A.: Effects of chronic arsenic exposure on hematopoietic function in adult mammalian liver. Environ. Health Perspec. 19: 209-214, 1977.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70200-05 LET
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) The Morphologic and Biochemical Effects of Toxic Metals		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: OTHER:	B. A. Fowler J. S. Woods C. M. Schiller C. A. Kimmel J. W. Ridlington N. G. Carmichael K. S. Squibb	Research Biologist Head, Biochem. Tox. Group Senior Staff Fellow Senior Staff Fellow Postdoctoral Fellow Visiting Fellow Postdoctoral Fellow LET NIEHS LET NIEHS LET NIEHS LET NIEHS LET NIEHS LET NIEHS
COOPERATING UNITS (if any) Department of Pathology, UNC Department of Environmental Hygiene, FDA, Washington, D.C. Karolinska Institute Department of Biochemistry, Duke		
LAB/BRANCH Laboratory of Environmental Toxicology		
SECTION Biochemical Toxicology Group		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 5.6	PROFESSIONAL: 4.6	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) When animals are exposed to different trace metals for prolonged time periods, each metal produces a biological response profile which specifically characterizes exposure to that metal. The objective of these studies is to assess and characterize response profiles based on a thorough understanding of subcellular mechanisms of metal toxicity and specifically to (1) define and correlate ultrastructural and biochemical responses <u>in vivo</u> which characterize exposure to toxic trace elements following prolonged exposure and (2) develop early, specific, and sensitive, biochemical <u>testing procedures</u> that may be used to evaluate human populations exposed to environmentally important trace elements. Specific metals and areas of interest include the biochemical effects of <u>cadmium, manganese, arsenic, mercury, selenium, and lead on mitochondrial structure and function</u> : Enzyme activities associated with <u>mitochondrial membranes</u> have been found to show early and pronounced changes in <u>mitochondria of rats and mice</u> following prolonged exposure to <u>arsenic</u> . Further development of these biochemical response profiles should permit a more basic understanding of the cellular mechanisms of metal toxicity and development of <u>metal-specific biochemical testing procedures</u> which accurately assess a biological response to these agents prior to overt toxicity.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Quantitative transmission electron microscopy; X-ray microanalysis; biochemical assays of mitochondrial respiration; and microsomal, cytosol, and mitochondrial enzymes. Sephadex and DEAE chromatography, electrophoresis, and amino acid analysis of metal binding proteins from mammals and marine shellfish. Tissue analysis for metals by atomic absorption spectroscopy and proton induced X-ray emission analysis.

MAJOR FINDINGS AND PROPOSED COURSE: (1) Arsenic. Combined ultrastructural and biochemical studies on liver mitochondria from rats and mice exposed to 0, 20, 40, or 85 ppm arsenate (As^{+5}) for six weeks produces in situ mitochondrial swelling. This ultrastructural effect was correlated with decreased state 3 respiration and respiratory control ratios for NAD but not succinate-mediated respiration. Further studies on the mechanism of this effect disclosed inhibition of pyruvate dehydrogenase activity and perturbation of the processes which regulate activity of the pyruvate dehydrogenase complex leading to an increase in the mitochondrial NAD/NADH ratio. Further ultrastructural morphometric/biochemical studies on rats exposed to the 40 ppm (As^{+5}) dose level disclosed an increase in the surface density of the inner membrane of hepatocyte mitochondria. Biochemical studies of mitochondria isolated from these animals showed an increase in mitochondrial metabolism of formaldehyde generated by microsomal oxidative dealkylation reactions. This effect is due in part to an increase in the specific activity of the NAD-dependent mitochondrial aldehyde dehydrogenase which is localized on the inner mitochondrial membrane. The observed increase in the activity of this enzyme appears to result from the increased mitochondrial NAD/NADH ratio and synthesis of mitochondrial membrane. Further studies are in progress. The effects of prolonged arsenate exposure on the hepatic mitochondrial heme biosynthetic pathway demonstrated an alteration of several enzymes of this pathway with a resultant uroporphyrinuria. These results are distinct from those observed with lead or methyl mercury exposure and suggest a selective effect of arsenic which may have utility in the pre-toxic detection of arsenic damage to exposed human populations.

(2) Cadmium. The long-term turnover and stability of Cd-metallothionein and translocation of Cd following two injections of 2 mg Cd/kg in rats was studied at 1,2,3,4 and 6 months following treatment; control and Cd-treated animals were pulse labelled with ^{35}S -cystine. The kidneys and livers were immediately removed and the Cd concentrations in heat-treated supernatant material from liver and kidney determined by atomic absorption spectrophotometry to ascertain the rate of Cd translocation from liver to kidney. A slow but definite translocation of Cd was observed. At each month interval, there was significant incorporation of ^{35}S -cystine into metallothionein while the (Cd):cystine ratio remained constant indicating that one exposure of Cd does not lead to permanent Cd-metallothionein but rather that the protein continues to turnover at a constant rate. Further studies on metallothionein from the

four to six month animals demonstrated that both A and B forms were present in relatively constant amounts showing similar turnover rates and stabilities for either form with time. The synthesis, composition, and turnover of cadmium-specific metal-binding proteins were also studied in oysters exposed to cadmium in sea water at a concentration of 0.1 mg/l for two weeks. The cadmium-binding protein isolated was found to have a molecular weight of about 10,000 and to be rich in dicarboxylic amino acids. It appears to be responsible for the rapid concentration of cadmium by oysters from sea water.

(3) Manganese. X-ray microanalysis studies have shown that crystalline concretions in kidneys of scallops contain high levels of manganese and are responsible for the bioaccumulation of this toxic element by these organisms. Further research is planned to study the role of the concretions in the uptake of other toxic metals such as cadmium by combining X-ray microanalysis with cell fractionation techniques and atomic absorption spectrophotometry to evaluate the relationship between total tissue concentrations of an element and its subcellular distribution.

(4) Lead. The ultrastructural and biochemical effects of chronic lead exposure were studied in renal tubule cells of rat kidneys. Rats were exposed prenatally to lead at dose levels of 0, 0.5, 5, 50 and 250 ppm lead (Pb) as lead acetate in drinking water and were continued on the respective regimens for 6 or 9 months after birth. Histopathological lesions in kidneys of these animals were characterized by cytomegaly/karyomegaly, nuclear inclusion body formation and increased numbers of iron-positive granules within proximal tubule cells. Ultrastructural studies disclosed mitochondrial swelling and the presence of increased numbers of lysosomes within renal proximal tubule cells. X-ray microanalysis performed on adjacent sections showed the relative intracellular distribution of lead within proximal tubule cells containing inclusions to be: inclusions>mitochondria>lysosomes>cytoplasm. Biochemical studies on renal mitochondria from animals in the same groups disclosed inhibition of mitochondrial respiration function for both succinate and NAD-linked substrates at 50 and 250 ppm Pb, dose levels at nine months. Mitochondrial δ -aminolevulinic acid synthetase and ferrochelatase were also inhibited at these doses and time periods.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies attempt to characterize and delineate the subcellular mechanisms of trace metal toxicity following prolonged exposure by combined ultrastructural and biochemical techniques. Once sufficient knowledge in this area is obtained, it may be applied to the development of metal-specific biochemical testing procedures which will accurately reflect a preclinical biological response to toxic trace metal exposure in human populations.

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Woods, J. S. and Fowler, B. A.: Renal porphyrinuria during chronic methyl mercury exposure. J. Lab. Clin. Med. 90: 266-272, August 1977.

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Fowler, B. A. and Vouk, V.: Bismuth. In Friberg, L., Nordberg, G. F., and Vouk, V. (eds.): Handbook on the Toxicology of Metals. Elsevier Pub. Co. (in press).

Fowler, B. A.: Indium. In Friberg, L., Nordberg, G. F., and Vouk, V. (eds.): Handbook on the Toxicology of Metals. Elsevier Pub. Co. (in press).

Tsuchiya, K., Ishinishi, N., and Fowler, B. A.: Arsenic. In Friberg, L., Nordberg, G. F., and Vouk, V. (eds.): Handbook on the Toxicology of Metals. Elsevier Pub. Co. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70210-01 LET															
PERIOD COVERED October 1, 1977 to September 30, 1978																	
TITLE OF PROJECT (80 characters or less) Effects of TCDD on the Disposition of Heme and Heme-Containing Proteins in Mammalian Tissues																	
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 45%;">K. T. Kitchin</td> <td style="width: 30%;">Staff Fellow</td> <td style="width: 10%;">LET</td> <td style="width: 10%;">NIEHS</td> </tr> <tr> <td>OTHER:</td> <td>J. S. Woods</td> <td>Head, Biochem. Tox. Group.</td> <td>LET</td> <td>NIEHS</td> </tr> <tr> <td></td> <td>N. M. Davidian</td> <td>Biochemical Toxicologist</td> <td>LET</td> <td>NIEHS</td> </tr> </table>			PI:	K. T. Kitchin	Staff Fellow	LET	NIEHS	OTHER:	J. S. Woods	Head, Biochem. Tox. Group.	LET	NIEHS		N. M. Davidian	Biochemical Toxicologist	LET	NIEHS
PI:	K. T. Kitchin	Staff Fellow	LET	NIEHS													
OTHER:	J. S. Woods	Head, Biochem. Tox. Group.	LET	NIEHS													
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COOPERATING UNITS (if any)																	
LAB/BRANCH Laboratory of Environmental Toxicology																	
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INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																	
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CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																	
SUMMARY OF WORK (200 words or less - underline keywords) These studies are designed to investigate the effects of <u>TCDD</u> on the biosynthesis and utilization of heme as well as the induction of <u>microsomal cytochromes</u> and related enzyme activities in <u>mammalian liver</u> . TCDD is known to produce <u>teratogenesis</u> and fetal lethality in mammals as well as <u>porphyria</u> in a variety of species. TCDD also increases the activation of various <u>exogenous carcinogenic hydrocarbons</u> and alters the metabolism of <u>endogenous steroid hormones</u> in mammalian tissues. The present studies demonstrate that TCDD induces the <u>de novo</u> synthesis of <u>apocytochrome P-448</u> which in turn combines with <u>endogenous heme</u> to form the spectrally and enzymatically distinct <u>holocytochrome P-448</u> . Time course studies following acute oral administration of TCDD demonstrate persistent elevation of hepatic microsomal aryl hydrocarbon hydroxylase (AHH) and <u>ethoxyresorufin O-deethylase</u> activities for as long as three months after a single treatment of TCDD. As little as <u>65 molecules of TCDD per hepatocyte</u> (0.002 µg/kg) caused a significant increase in AHH activity. These studies demonstrate a biological basis for the effects of TCDD at very low doses in mammalian liver.																	

PROJECT DESCRIPTION

METHODS EMPLOYED: Livers and kidneys from female rats are obtained after acute or chronic administration of TCDD. Microsomes are isolated from livers and kidneys after subcellular fractionation by differential centrifugation. Data are analyzed using commonly accepted statistical methods.

These experiments study the time course, dose response relationship, pharmacokinetics, and mechanism of TCDD induction of hepatic cytochrome P-448 and aryl hydrocarbon hydroxylase (AHH) activity. Animals are housed singly in hanging cages after the i.p. (0-24 hours) or oral (1 day- 6 months) administration of TCDD. Ten different doses of TCDD ranging from 0.0006 to 20.0 µg/kg are used to determine the dose-response relationship of TCDD's induction of cytochrome P-448 and AHH activity. Carrier free [1,6-³H]-TCDD is given to animals to estimate the hepatic content of TCDD by the biological material oxidation method. After incubation of whole liver homogenates with hemin, the *in vitro* reconstitution of cytochrome P-448 levels and AHH activity in samples from control and treated animals are measured by spectral and enzymatic methods. Protein synthesis inhibitors and hematotoxic agents such as cobalt chloride are used to establish the effect of TCDD on *de novo* induction of cytochrome P-448 and AHH activity.

MAJOR FINDINGS AND PROPOSED COURSE: Results of recent studies indicate that TCDD (2 µg/kg) does not directly affect hepatic heme biosynthesis and does not increase the activity of δ-aminolevulinic acid synthetase, the rate limiting enzyme in that process, at any of the time points examined. In contrast, a 45 fold increase in the activity of the cytochrome P-448-mediated aryl hydrocarbon hydroxylase was observed seven days after TCDD administration. Protein synthesis inhibitors, but not heme depleters, completely prevented the induction of new cytochrome P-448 and increased AHH activity. *In vitro* reconstitution of total cytochrome P-450 levels and AHH activity were observed after adding hemin to whole liver homogenates. These *in vitro* studies showed that 600% greater reconstitution of total microsomal apocytochromes with hemin and 450% greater increases in AHH levels occurred in liver homogenates of heme-depleted and TCDD-induced rats as compared to controls. These studies indicated that TCDD induces the synthesis of apocytochrome P-448 which combines with heme to form new cytochrome P-448 and increased AHH activity. Low dose studies revealed that the TCDD induced AHH activity at a dose of 0.002 µg/kg, which corresponds to about 65 molecules of TCDD per liver cell. These biochemical alterations in the livers of TCDD-treated animals are still present three months after administration. Microsomal oxidative homeostasis returns by six months following TCDD administration. The novel, light stable, non-carcinogenic substrate 4,4'-dimethylbiphenyl, which forms a single fluorescent metabolite, was used as an aryl hydrocarbon hydroxylase substrate. Future studies will determine the structure of the hepatic microsomal metabolite of 4,4'-dimethylbiphenyl. This substrate will be used to develop a rapid radio-metric assay for aryl hydrocarbon hydroxylase. This new AHH assay could

be more dependable and safer than the use of benzo(α)pyrene, a light unstable carcinogen, as a substrate.

A mixed function oxidase reconstitution system using partially purified microsomal hemoproteins (cytochrome P-450 and P-448) and microsomal membranes having low endogenous hemoprotein-mediated catalytic activity will be developed. Renal and hepatic microsomal preparations will be used. Cumene hydroperoxide, an in vitro hemoprotein depletor, can be used to further lower hemoprotein enzyme activity. Cytochrome P-448 and P-450 will be isolated from hepatic microsomes of TCDD and phenobarbital treated rats, respectively, by column chromatography and calcium phosphate gel absorbtion and elution.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Chronic exposure of rats to TCDD is known to increase the number of tumors of the respiratory system and liver while decreasing the number of tumors found in endocrine organs such as pituitary, uterus, mammary gland and adrenal glands. Knowledge of the mechanism by which environmentally persistent, highly toxic, acneogenic and teratogenic chlorinated hydrocarbons such as TCDD selectively alter renal and hepatic mixed function oxidase activity is essential to the development of a biochemical basis for both understanding and preventing chronic organ toxicity. The completed studies have shown that TCDD's effects on hepatic biochemical parameters persist for longer (3-6 months) than was previously known. TCDD induces specific hepatic oxidative capabilities at doses as low as 0.002 ug/kg, 1/100 the previously known lowest dose effect in mammals. This process requires de novo synthesis of apocytochrome P-448. Proposed studies will investigate the interaction and increased catalytic ability of various microsomal membranes after in vitro incubation with partially purified hepatic cytochromes. In addition a safer in vitro assay for aryl hydrocarbon hydroxylase, an enzyme which mediates the activation of many chemical carcinogens, will be developed using 4,4'-dimethylbiphenyl as a substrate.

PUBLICATIONS

Kitchin, K. T. and Woods, J. S.: 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) induction of aryl hydrocarbon hydroxylase in female rat liver. Evidence for de novo synthesis of cytochrome P-448. Molecular Pharmacology (in press).

OAK RIDGE NATIONAL LABORATORY, U.S. ENERGY RESEARCH AND DEVELOPMENT
ADMINISTRATION - Oak Ridge, Tennessee 37830
(ERDA 40-524-75)

TITLE: Environmental Teratology Information Center (ETIC)

CONTRACTOR'S PROJECT DIRECTOR: John S. Wassom, B.Sc.

PROJECT OFFICER (NIEHS): Robert E. Staples, Ph.D.

DATE CONTRACT INITIATED: August 15, 1975

CURRENT ANNUAL LEVEL: \$300,000

PROJECT DESCRIPTION

OBJECTIVES: To establish a teratology information center.

METHODS EMPLOYED: (1) To collect reprints of all publications that contain data pertinent to determination of the teratogenic potential of environmental agents or conditions to which man is exposed; and (2) to use facilities available at Oak Ridge National Laboratories to extract specific information from relevant references and to develop methodology permitting computer storage and retrieval of this information.

MAJOR FINDINGS AND PROPOSED COURSE: Initial effort involved identification and accumulation of references to studies conducted on warm-blooded animals whether test results were positive or negative. To date, more than 14,000 references have been identified and entered into ETIC, a computerized system.

The chemical dictionary that was developed now lists more than 3,000 chemical agents in the ETIC system. Information for each chemical listed includes the unique Chemical Abstract Service Registry Number (CAS NO), molecular formula, synonyms, trade names, generic names and Wisswesser Linear Code.

Each reference in ETIC is indexed to permit search by agent or species tested and by author. Article titles also can be searched for key words. Additionally, chemical agents are indexed with the CAS NO which enables studies on any given chemical agent to be identified whether the author used the trade name, synonym, etc. in his writing.

Information contained in ETIC can now be accessed as a subfile of TOXLINE (Toxicology Information On-line), which is available through the National Library of Medicine, or, as a subfile of RECON, which is available through Oak Ridge National Laboratories. These files are updated periodically. Earlier

studies were located largely through scanning teratology files made available by individual teratologists.

The more recent references are located through ASCA, by scanning about 30 key journals, and by automated scanning of available computer tapes from large multidisciplinary data bases (e.g. Chemical Abstracts) via a teratology profile.

Names and addresses of members of the American, European, and Japanese Teratology Societies have been entered on tape. These listings are updated when possible, and are used to contact scientists. Their cooperation is sought to keep ETIC informed of current studies and to provide new references more rapidly.

The advisory committee, composed of three individuals with varied expertise, continue to provide guidance to the Project Officer toward development of ETIC.

The proposed course is to continue the input of about 300-400 references per month, to include information from non-published or current studies to be found in government agencies, in academic as well as in private industry and to begin the creation of a Teratology Data Bank by extraction of additional information from each article located to date.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Because the field of teratology is relatively new and because teratology data on chemical and other agents are widely scattered in the literature, there is an urgent need to bring together data pertaining to this area. To this end, ETIC has organized much of the literature about the teratogenic effect of environmental agents into a computerized system. The extracts to be prepared from each reference and entered into a Teratology Data Bank will make additional information available to users, i.e., dose levels tested, route of administration, vehicle used, number and type of malformations observed, number of animals tested, etc. These systems may be used to minimize unplanned duplication of tests, to aid in determination of the teratogenic status of individual agents, and to establish priorities in the planning of future investigations.

RESEARCH TRIANGLE INSTITUTE - Research Triangle Park, NC 27709
(N01-ES-6-2127)

TITLE: Teratogenic Potential of Environmental Agents

CONTRACTOR'S PROJECT DIRECTOR: Thomas Marks, Ph.D.

PROJECT OFFICER (NIEHS): Robert E. Staples, Ph.D.

DATE CONTRACT INITIATED: June 1, 1976

CURRENT ANNUAL LEVEL: \$255,026

PROJECT DESCRIPTION

OBJECTIVES: To determine the teratogenic potential in animals of environmental agents.

MAJOR FINDINGS AND PROPOSED COURSE: The study comparing tap water to purified tap water described in last year's annual report, was completed and a manuscript has been drafted for internal review.

The polychlorinated biphenyl isomers tested to date for teratogenic potential in mice are 3,4,5,3',4',5' HCB; 3,4,3',4' TCB; and 4,4' DCB. Additional isomers under study are 2,4,6,2',4',6' HCB and 3,4,3',4' tetramethylbiphenyl.

Other agents currently being tested are formaldehyde, potentiated acid glutaraldehyde (Sonacide®), n-hexane phenacyclidine, and epichlorhydrin.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Most environmental agents have not been tested for teratogenic potential. This contract was established to provide the capability to test agents of high priority either because of possible exposure to man or because the information obtained can be used to aid our understanding of how the process of mammalian development can be altered to result in malformed individuals. One-third of the resources of this contract are used to complete experimentation begun at NIEHS.

THE DOW CHEMICAL COMPANY - Midland, Michigan 48640
(NIH-NIEHS-73-2102)

TITLE: Effect of Environmental Components on Reproduction and Ontogenesis

CONTRACTOR'S PROJECT DIRECTOR: F. J. Murray, Ph.D.

PROJECT OFFICER (NIEHS): Robert E. Staples, Ph.D., Research Physiologist
Teratology Group, LET

DATE CONTRACT INITIATED: February 5, 1973

CURRENT ANNUAL LEVEL: \$300,000

PROJECT DESCRIPTION

OBJECTIVES: (1) To determine the teratogenic potential in animals of environmental components when administered singly or in combination; (2) to determine species differences in response to teratogens and to study the etiology of such differences; and (3) to investigate the mechanism of action of teratogens and to improve experimental testing and extrapolation to man.

MAJOR FINDINGS AND PROPOSED COURSE: Sulfuric acid aerosol was tested for teratogenic potential in mice and rabbits at dosage levels of 0, 5 and 20 mg/m³ for 7 hr. per day on Days 6 through 15 and 6 through 18, respectively. The aerosol was well tolerated by the dams. In mice, maternal liver weight and fetal body weight were significantly reduced at the 20 mg/m³ dose level, but to date no other H₂SO₄-related embryotoxicity or teratogenicity was obtained. In rabbits, maternal body weight gain was significantly reduced at the high dose but no other adverse maternal or fetal effects were noted. The fetal skeletons of both species will be examined.

Tetrachloroacetone, a persistent component of chlorinated drinking water, was administered by gavage to mice from Days 6 through 15 and to rabbits from Days 6 through 18 of gestation. Dose levels tested were 0, 5, 15, and 50 mg/kg/day for mice and 0, 1, 3, 10 mg/kg/day for rabbits. Considerable maternal toxicity resulted from the 50 mg/kg/day dosage in mice, but no toxicity was seen at the dose level of 5 mg/kg/day. A significant increase in cleft palates and developmental delay was noted in the fetuses of the 15 and 50 mg/kg/day dose group. However, a positive teratogenic response was not noted in the group given tetrachloroacetone at the 5 mg/kg/day dose level. In rabbits, no adverse effects were noted in the dams or their fetuses.

Hexachlorocyclopentadiene (HCCP), a common chemical intermediate used in the manufacture of several chlorinated pesticides (e.g. Mirex, Kepone, Aldrin, and Dieldrin), was given by gavage to mice and rabbits. Since maternal deaths resulted in both species at dose levels of 250 and 500 mg/kg/day, the largest dose level studied for both species was 75 mg/kg/day. As no adverse effects

were seen in the mouse, study of larger doses are anticipated. In the rabbit a significant decrease in maternal weight gain was measured in the group given HCCP at 75 mg/kg/day, and maternal deaths were observed; however, no adverse effects were noted on embryo and fetal development.

Three manuscripts (on ethanol, carbaryl, and chloroform) have been submitted to journal editors for publication, another on carbon monoxide is being reviewed "in-house", and manuscript drafts on Dichlorvos and on Dichlorvos with chemical modifiers have been prepared. A manuscript draft on phenobarbital, SKF-525A, and piperonyl butoxide, and a second one on carbaryl with phenobarbital and piperonyl butoxide are being prepared.

The proposed course is to determine the teratogenic potential of industrial dyes and the effects of epichlorohydrin on fertility. Manuscript drafts are to be completed on all remaining compounds studied at this writing by September, 1978, when this contract will be terminated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Most environmental agents have not been tested for teratogenic potential. These studies were undertaken to provide such information on a variety of agents to which man is exposed. Since it is known from studies in laboratory animals that combined administration of two teratogens can potentiate teratogenic response, combinations of non-teratogenic agents were tested to determine whether conditions of increased sensitivity to the developing embryo might be revealed. This information will serve in the formulation of a rational basis for evaluation of the potential hazards to human development produced by the use of and exposure to these chemicals.

PUBLICATIONS

Schwetz, B. A., Nitschke, K. D. and Staples, R. E.: Cleft palates in CF-mice after deprivation of water during pregnancy. Toxicol. Appl. Pharmacol. 40, 2: 307-316, 1977.

Murray, F. J., Schwetz, B. A., Crawford, A. A., Henck, J. W., and Staples, R. E.: Teratogenic potential of sulfur dioxide and carbon monoxide in mice and rabbits. Proceedings of 17th Hanford Biological Symposium on "Developmental Toxicology of Energy-Related Pollutants", Richland, Washington, October 17-19, 1977 (in press).

ENDOCRINE LABS OF MADISON - Madison, Wisconsin 53707
(NIEHS N01-ES-6-2111)

TITLE: Effects of Environmental Agents on Male and Female Reproductive Tract Function

CONTRACTOR'S PROJECT DIRECTOR: James Davis, Ph.D.

PROJECT OFFICERS (NIEHS): G. W. Lucier, Ph.D., Head, Develop. Toxicol. Group
Developmental Toxicology Group, LET
J. A. McLachlan, Ph.D., Developmental Toxicologist
Transplacental Toxicology Group, LET

DATE CONTRACT INITIATED: March 20, 1976

CURRENT ANNUAL LEVEL: \$245,000

PROJECT DESCRIPTION

OBJECTIVES: A series of experiments are described which will provide information on the effects of 36 selected xenobiotics (10 per annum) on the fertility of mice (male and female) following prenatal or postnatal exposure, and determine if levels of reproductive hormones have deviated from the norm or if a pathologic condition exists in mice whose reproductive capacity has been altered. Information will be obtained on the possible developmental toxicity of these chemicals, including transplacental carcinogenesis.

METHODS EMPLOYED: The approach that we are using to assess transplacental toxicity involves three phases of investigation. Phase I involves a preliminary screen to determine the "critical dose", which is defined as the highest dose that does not cause significant fetotoxicity or teratogenicity. Pregnant CD-1 mice are used as the test animal and chemicals are administered on days 10 through 16 of gestation. When generating the value for the "critical dose," we attempt to collect meaningful teratogenic and fetotoxic data so that maximum experimental information can be obtained from the protocol. In the teratogenicity studies, two-thirds of the fetuses (19 days of gestation) from each litter are fixed in 95% methyl alcohol and examined for skeletal alterations. One-third of the fetuses are preserved in Bouin's fixative, sectioned, and examined for internal anomalies. Autopsy data include determinations of corpora lutea, implants, resorptions, viable fetuses, fetal body weight, and sex ratio.

Dose schedules for animals used in Phase II are; controls, one-half of the critical dose, and one-tenth of the critical dose. If no "critical dose" is established, doses of one-half and one-tenth the LD₅₀ are employed. In Phase II studies, 24 pregnant females are used in each group and are administered vehicle or vehicle plus chemical on days 10 through 16 of gestation. One group of animals receives a dose of DES (10 mg/kg on days 10-16), which

significantly reduces fertility of both male and female offspring and this group is used as a positive control. Parameters evaluated in the initial Phase II studies include fertility, gestation, viability, survival, and lactation indices. Necropsy examinations of F_1 males are made at eight weeks, six months, 12 months, and a terminal necropsy is also performed. F_1 males are examined at seven weeks and again at the termination of the experiment. Tissues examined for histological alterations include liver, kidney, lung, small intestine, spleen, thymus, pituitary, adrenal, thyroid, parathyroid, gonads, uterus, epididymis, cervix, vas deferens, vagina, seminal vesicles and prostate. All tissues are checked for gross lesions. Fertility of at least 20 F_1 males are evaluated at eight weeks and again at eight months of age. Two virgin females (3-10 weeks old) are placed with each male every five days for 25 days (total of 10 females per male). Twelve days after removal, the females are weighed, laparotomized and the number of fetuses (alive, dead, or abnormal) and resorptions recorded. Fertility of F_1 females are determined by a lifetime forced-breeding study. Fifty F_1 females are selected randomly from each treatment group and each female is cohabited with one proven breeder male. The F_1 females remain with the males and are allowed to deliver at term. The number of F_2 young (alive and dead) are recorded and the young examined for gross anomalies. Litters are examined again on days 4 and 20. New breeder males are replaced at 180 - day intervals. Plasma samples are taken prior to necropsy for analysis of progestins, androgens, and estrogens in groups of treated animals exhibiting deficits in reproductive tract function. The estrous cycle is monitored in eight week old F_1 females.

Although emphasis is placed on reproductive tract function of F_1 offspring, it would seem wasteful and narrow-minded to ignore manifestations of impaired organ function of other systems. Accordingly, the offspring are monitored for behavioral parameters, immunological competence, transplacental carcinogenesis, and cardiovascular function.

The objectives of Phase III are to determine dose-response relationships of chemicals exhibiting transplacental toxicity, to elucidate mechanisms of toxicity, to characterize structure-activity relationships, and to define the role of perinatal pharmacokinetics in transplacental toxicity. Of course, the protocols for Phase III studies cannot be developed until at least portions of Phase I and Phase II studies are completed. Many of the Phase III studies are conducted at the NIEHS.

MAJOR FINDINGS AND PROPOSED COURSE: Chemicals investigated or planned for study include DES, TCDD, DES + TCDD, Zearanol, Zearalenone, Mirex, Kepone, DDE, Methoxychlor, Hexabromobiphenyl (6-BB), Hexachlorobiphenyl (6-CB), Tetrachlorobiphenyl (4-CB), DMBA, 3-Methylcholanthrene, Benzpyrene, Dichloropropene, Phosphamidon, Dicrotophos, Atrazine, Aldicarb, and Carbaryl. Completed protocols for reproductive capacity following treatment with these chemicals are not available since these are lifetime studies. The fetotoxic and/or teratogenic dose has been established or will be established for each of these compounds in the CD-1 mouse. For example, Zearanol, a synthetic Mycotoxin analog, was shown to cause skeletal abnormalities at a dose of 300 mg/kg/day on days 10-16 of gestation. The estrogenic potency of this compound was determined

to be approximately 1/600 that of DES. However, reproductive capacities of these offspring were unaffected. Localization of zearanol/zearalenone in specific fetal tissues will be performed by radioimmunoassay procedures.

Marked and unusual behavioral changes were observed in offspring from 4-CB treated (32 mg/kg/day) pregnant mice by two to three weeks after parturition. Increased locomotor activity and general restlessness were noted in approximately half of the surviving litters. General symptoms included characteristic jerking or rotational movement of their heads. There were also episodes of constant circling movement which seemed to be initiated by a loud noise. All offspring in an affected litter exhibited behavioral alterations and the symptoms were evident at least through eight months of age. Affected animals gained weight at approximately the same rate as controls and the survival rates of rotating and non-rotating offspring in the treated group were not different. Moreover there was no correlation between hydronephrosis and behavioral alterations and the occurrence of hyperactivity was not sex-related. The complexity of the symptomology indicates damage to the central nervous system. Although conventional histological techniques did not find microscopic alteration, electron micrographs revealed CNS outgrowths into the peripheral nervous system. This lesion resembles those observed in the Werdnig-Hoffman syndrome. Foster mother experiments demonstrated that the effect is related to prenatal exposure to 4-CB.

Tissue distribution studies of ¹⁴C-4-CB in CD-1 mice revealed some important findings. We observed that only very low levels of ¹⁴C-4-CB were detected in weanlings following exposure to ¹⁴C-4-CB via both placental transfer and suckling. Radioactivity was not detectable in eight week-old offspring indicating that behavioral alterations occur in the absence of the chemical. These studies also revealed that newborn tissues had, in general, higher 4-CB concentrations at parturition than did maternal tissues. This finding may help explain the occurrence of fetotoxicity and teratogenicity in 4-CB treated groups whereas no such effects were found in 6-CB and 6-BB treated animals.

It also appears that mice exposed to 4-CB prenatally have decreased reproductive capacity.

Studies with the polycyclic aromatic hydrocarbon (PAH) dimethylbenzanthracene (DMBA), an environmental carcinogen, have shown that gestational treatment with as little as 1.25 mg/kg results in absence of germ cells in both male and female offspring at seven weeks of age. Other abnormalities were not noted; these effects could not be induced with other PAHs, 3-methylcholanthrene or benzyrene at doses 50 fold higher than that for DMBA.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Environmental chemicals can cause reproductive toxicity in mammals which is manifested in decreased fertility. However, these chemicals might affect male and female reproductive processes differently since oogenesis is an entirely prenatal event and spermatogenesis occurs throughout adult life in most

eutherian mammals. The possibility exists that lesions of the reproductive tract which occur in utero might result in long term impairment of the reproductive tract in females and have a totally different effect on the male reproductive process.

The objective of the proposed study is to determine the effects of environmental chemicals on the reproductive capacity of male and female mice. Reproductive capacity is to be evaluated by the repetitive forced breeding method for females and by a serial mating technique for males. Since those animals are held for 18 months, developmental toxicities of other organ systems are noted including the CNS, immune and cardiovascular systems. The potential for transplacental carcinogenesis is also noted.

These studies are done in such a way as to support existing programs in the Laboratory of Environmental Toxicology concerned with developmental toxicity and transplacental toxicity. The purpose of the contract is two-fold; to identify environmental chemicals that have potential developmental toxicity emphasizing reproductive tract lesions; and to identify chemicals to Intramural scientists that are toxic and need to be studied from a mechanistic viewpoint.

PUBLICATIONS

Lucier, G. W., Davis, G. J. and McLachlan, J. A.: Transplacental toxicology of the polychlorinated and polybrominated biphenyls. Proceedings of the Conference on the Developmental Toxicology of Energy Related Pollutants. (in press).

FISHERIES RESEARCH INSTITUTE - Univ. of Washington, Seattle, Washington
(NIH-N01-ES-7-2101)

TITLE: Carcinogenic Effects of Petroleum Hydrocarbons on Selected Marine and Estuarine Organisms

CONTRACTOR's PROJECT DIRECTOR: Sam P. Felton and Bruce S. Miller

PROJECT OFFICERS (NIEHS): J. R. Bend, Ph.D., Visiting Scientist
Laboratory of Pharmacology
B. A. Fowler, Ph.D., Research Biologist
Laboratory of Environmental Toxicology

DATE CONTRACT INITIATED: December 16, 1976

CURRENT ANNUAL LEVEL: \$92,556

PROJECT DESCRIPTION

OBJECTIVES: To compare incidence of tumors, particularly epidermal papillomas, in exposure and control groups of selected flatfish species in response to exposure to benzo(a)pyrene (BaP) and/or its electrophilic derivatives and to qualitatively examine the histopathology of comparable tumors occurring in both exposure and control groups.

METHODS EMPLOYED: Static and flowing sea water systems with aquaria and sand coated with BaP. Monitoring of BaP concentrations by gas chromatography, assay of aryl hydrocarbon hydroxylase (AHH), examination of fish tissue by routine histopathological procedures.

MAJOR FINDINGS AND PROPOSED COURSE: Progress to date has centered on completing the glass/teflon seawater exposure systems, refining AHH assay procedures for flatfish and developing the necessary analytical and pathology techniques for assessment of fish tissues. Seasonal variation in AHH activity was observed with fall and winter months being periods of low activity. Baseline toxicity studies disclosed that BaP produces mortality in juvenile flatfish with renal and intestinal damage as the major histopathological findings. Studies concerning long-term exposure to BaP, induction of AHH and evaluation for tumors are now in progress.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies should provide fundamental information about the metabolism and biological responses to a carcinogenic petroleum hydrocarbon of marine organisms consumed as food. Results of the study should specifically yield useful data on the relationship between AHH activity and the carcinogenic susceptibility of marine organisms.

Proposed course is to expose flatfish to BaP and examine the relationship between AHH activity and carcinogenesis in these species.

MIDWEST RESEARCH INSTITUTE - Kansas City, Missouri 64141
NIEHS-72-2084

TITLE: Evaluation of the Environmental Toxicant, Vinyl Chloride Monomer

CONTRACTOR'S PROJECT DIRECTOR: C. C. Lee, Ph.D.

PROJECT OFFICER (NIEHS): J. S. Woods, Ph.D., Head, Biochemical Toxicol. Group
Biochemical Toxicology Group, LET

DATE CONTRACT INITIATED: December 31, 1974

CURRENT ANNUAL LEVEL: \$70,000

PROJECT DESCRIPTION

OBJECTIVES: This contract concerns the toxic effects of vinyl chloride monomer (VCM) and vinylidene chloride monomer (VDM) in mammals during low level, long term inhalation exposure. The specific objectives of these studies can be summarized as follows: (1) to determine nonmalignant biochemical and pathological alterations in specific organ functions of rats and mice during exposure to VCM and VDM, (2) to identify, innumerate and characterize malignant alterations occurring in VCM- and VDM-exposed animals and to ascertain time-to-tumor development, and (3) to establish VCM and VDM response profiles for use in high to low dose, animal to human extrapolation studies.

METHODS EMPLOYED: Inhalation exposure techniques, conventional chromatographic procedures, U.V., visible and I.R. spectroscopy, histology, pathology, hematology, clinical blood chemistry, immunological techniques, and cytogenic analyses.

MAJOR FINDINGS AND PROPOSED COURSE: Vinyl chloride monomer and vinylidene chloride studies were initiated in September 1975. Rats and mice of both sexes have been exposed for 6 hours per day, 5 days per week to 50, 250, or 1000 ppm vinyl chloride or to 55 ppm vinylidene chloride. Hematologic, pulmonary, immunologic, biochemical, and cytogenic analyses conducted on exposed animal tissues demonstrated no adverse changes in non-malignant clinical laboratory parameters in rats of either sex and no apparent changes in these data in mice with the exception of increased pulmonary macrophage counts at 1000 ppm VCM in males. In contrast, numerous malignancies have appeared in both species after 12 months exposure to both VCM and VDM and 10 months recovery time. In rats exposed to VCM, hemangiosarcomas of the liver, lung, skin and adrenal gland, as well as tumors of the mammary gland and other sites were observed. Major tumors found in mice exposed to VCM included bronchiolar adenoma, hepatic hemangiosarcoma, various mammary gland tumors and malignant lymphoma. Tumor incidence was dose-related in most cases and was greater in females of both species. In animals exposed to VDM, one subcutaneous hemangiosarcoma of the skin was seen in rats, and three tumors of the lung and/or liver occurred in mice. Proposed studies are to complete the specified objectives of the

contract in regard to the assessment of chronic toxic effects of VCM and VDM and to utilize these results in the design of models for the assessment of risk of exposure to these agents in human populations.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These investigations will establish the effect and nature of chronic toxicities of vinyl and vinylidene chloride using rats and mice. This information will serve to form a rational basis for evaluation of the potential human health hazards produced by exposure to these chemicals.

PUBLICATIONS

Winston, J. M., Lee, C. C., Bhandari, J. C., Dixon, R. L., and Woods, J. S.: A study of the Carcinogenicity of Inhaled Vinyl Chloride and Vinylidene Chloride in Rats and Mice. Proceedings of the First International Congress on Toxicology (G. Plaa, Ed.) Academic Press, NY, 1977 (in press).

Lee, C. C., Bhandari, J. C., Winston, J. M., House, W. B., Peters, P. J., Dixon, R. L. and Woods, J. S.: Inhalation toxicity of vinyl chloride and vinylidene chloride. Environ. Health Perspec. 21: 25-32, 1977.

Lee, C. C., Bhandari, J. C., Winston, J. M., House, W. B., Dixon, R. L. and Woods, J. S.: Carcinogenicity of vinyl chloride and vinylidene chloride. J. Tox. Environ. Health 4: 15-30, 1978.

NATIONAL MARINE FISHERIES SERVICE - Beaufort, North Carolina 28516
(Interagency Agreement Between NIEHS & NOAA)

TITLE: The Physiological Effects of Arsenic, Cadmium, and Copper on Marine Shellfish

CONTRACTOR'S PROJECT DIRECTOR: David W. Engel, Ph.D.

PROJECT OFFICER (NIEHS): B. A. Fowler, Ph.D., Research Biologist
Biochemical Toxicology Group, LET

DATE CONTRACT INITIATED: July 23, 1975

CURRENT ANNUAL LEVEL: \$50,000

PROJECT DESCRIPTION

OBJECTIVES: This study was undertaken to determine the physiological, biochemical, and ultrastructural effects of arsenic, cadmium, and copper on tissues of marine shellfish in comparison with those previously observed in mammals. Evaluation of pharmacokinetic and toxicological differences in comparison to similar doses of Cd administered as mammalian cadmium-thionein, oyster cadmium-binding protein or Cd²⁺ is to be accomplished.

METHODS EMPLOYED: Flowing seawater systems with continuous trace element injection, atomic absorption spectroscopy, radiotracer analyses, histological and ultrastructural techniques, cellular respiration studies, proton-induced X-ray emission analyses.

MAJOR FINDINGS AND PROPOSED COURSE:

Effects of Salinity on Cadmium Uptake by Shellfish. On the basis of studies conducted to date it is clear that salinity plays a crucial role in determining the bioavailability, uptake, and toxicity of metals such as cadmium. Low salinities such as those found in estuaries seem to alter bioavailability. Further studies to examine the effects of low salinity (10‰) on cadmium uptake by shellfish and to relate these findings to tissue respiration changes, ultrastructural and X-ray microanalytical studies and binding to the oyster cadmium protein are in progress.

Effects of Copper and Cadmium on Shellfish. Experiments were conducted to determine the effects of copper and cadmium on the gill tissue of oysters, *Crassostrea virginica*. Metal accumulation and oxygen consumption, were related to ultrastructural alteration of gill tissue and the presence of metal-binding proteins for cadmium or copper. The oysters were exposed to either 50 or 100 ppb copper, or 100 or 600 ppb cadmium continuously for up to 14 days in a flowing seawater system. Gill tissue from exposed oysters accumulated both copper and cadmium as a function of time of exposure. Significant

increases in the rate of oxygen consumption were observed in the 100 ppb copper exposure and 600 ppb cadmium groups. Ultrastructural evaluation of gill tissue from oysters exposed to 100 ppb copper or 100 ppb cadmium for 14 days showed that only copper caused discernable intracellular changes. Exposure to 100 ppb cadmium resulted in extensive cadmium binding to a low molecular weight protein which was not found in copper-treated animals. At the 600 ppb cadmium exposure level, binding of the metal to higher molecular protein species was observed. Further ultrastructural/biochemical studies on the mechanisms of copper and cadmium toxicity to shellfish are in progress.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies will provide a basis for evaluating mechanisms of uptake and cellular responses to chronic low level trace metal exposure in tissues of relatively simple organisms in comparison to those observed in mammals.

Data generated from this effort should also prove useful in evaluating differences in trace metal pharmacokinetics and toxicity following ingestion of metal contaminated marine shellfish. The future course of the project will involve the specified objectives of this interagency agreement in relation to understanding the subcellular mechanisms of trace element uptake and toxicity in marine organisms in comparison to mammals. This arrangement also provides a mechanism for estimating potential human risk following ingestion of metal contaminated marine shellfish.

PUBLICATIONS

Engel, D. W. and Fowler, B. A.: Accumulation and biological effects of copper and cadmium on marine shellfish. Program review proceedings of "Environmental Effects of Energy Related Activities on Marine Estuarine Ecosystems. Interagency energy environment research and development program report. EPA-600/7-77-111, pp. 45-56, October 1977.

Engel, D. W. and Fowler, B. A.: Copper and Cadmium-Induced Changes in the Metabolism and Structure of Molluscan Gill Tissue. In: Marine Pollution; Functional Responses. W. B. Vernberg and F. J. Vernberg (eds.) Academic Press, 1978 (in press).

Engel, D. W. and Fowler, B. A.: Factors influencing the accumulation and toxicity of cadmium to marine organisms. Environ. Health Perspec. 1979 (in press).

LABORATORY OF PHARMACOLOGY

LABORATORY OF PHARMACOLOGY Summary Statement

The Laboratory of Pharmacology initiates and carries out research programs that are primarily concerned with (1) mechanisms by which environmental pollutants and effectors cause toxicity, (2) the development of better test systems for detecting and evaluating early toxic effects of chemicals occurring as environmental contaminants, and (3) extrapolating from animal test systems across species and to man. The Laboratory is organized into two formal sections, the Toxication-Detoxication Section and the Marine Pharmacology and Biomedicine Section.

A. Toxication-Detoxication Section

This program area is concerned with the role of chemical metabolism in the mediation of toxicity such as tissue damage. Metabolic processes leading to more toxic products (activation) may be at least partially counteracted by metabolic processes which form less toxic products (deactivation). This balance of toxication and detoxication pathways may be at least partially responsible for the target organ toxicity associated with specific classes of xenobiotics and may be upset by previous or concomitant exposure to pollutants or drugs, by disease states, or by hormone changes. Some of these activation and deactivation enzymes are also dependent upon animal age, sex, and species.

Chemical-metabolizing enzyme systems have been and are being characterized in tissues that serve as interfaces with the environment and its pollutant load (skin, intestine, and lung), in liver which serves as the major tissue for the biotransformation for most organic xenobiotics, and in some steroidogenic tissues necessary for maintaining homeostasis and reproduction capability (adrenal, testis, and ovary). Characterization of the enzymes in these cases includes substrate specificity, inhibitor effects, ability to be induced or stimulated by previous exposure to environmental contaminants, and separation and purification of individual constituents of multicomponent systems.

B. Marine Pharmacology and Biomedicine Section

This program area conducts studies on the uptake, distribution, metabolism, and excretion of pollutants by various marine species, and the role of metabolism in the storage and the chemical form of the accumulated xenobiotics in these species. The major emphasis is on how, why, and where marine species accumulate pollutants which have potential for harm to man and whether mixtures of pollutants may lead to accumulation of more toxic forms or higher levels of pollutants than single chemical exposure. Effect of water temperature on metabolic, storage, and

excretion processes is being studied. The pharmacokinetics of pollutant distribution in selected marine species is also being determined and compared with mammals (with the Laboratory of Pharmacokinetics). Particular emphasis is being placed on studying formation and further metabolism of chemically reactive metabolites (e.g., arene oxides from polycyclic hydrocarbons which are constituents of crude oil). Stress is placed on the cytochrome P-450 containing MFO systems and other hydrolytic and conjugating pathways, including effects of pre-exposure to environmental contaminants on these enzyme systems. Where biologically significant induction is observed, the induced system will be characterized in considerable detail. Since most marine species have lesser abilities than mammalian species to oxidize chemicals, the nonoxidative (e.g., conjugating) enzyme systems are being studied in several tissues of marine species. The Section is also involved in developing a national program (NIH focus) in marine pharmacology as directly applicable to human health problems (e.g., models for human disease and sources of toxic materials, such as carcinogens and mutagens for man).

Personnel

Dr. J. R. Fouts (Scientific Director) returned as Chief, Laboratory of Pharmacology, during this fiscal year. Additions to the Laboratory were: Staff Fellow -- Shelley Slaughter (with Dr. Fouts); Chemist -- Charles Scott (with Dr. Slaughter); Biological Aid -- Julie Angerman (with Dr. Pritchard); Visiting Fellows -- John Plummer (with Dr. Bend) and Peter Little (with Dr. James). Individuals leaving the Laboratory of Pharmacology included Dr. R. Chhabra (who transferred to the Research Resources Program), Dr. Chris Miranda, and Mr. Robert Nardone.

Other Activities

Dr. J. R. Fouts: Adjunct Professor, Dept. Pharmacology, School of Medicine, University of North Carolina - Chapel Hill; Adjunct Professor, Dept. Entomology, School of Life Sciences, North Carolina State University, Raleigh; Visiting Professor, Depts. Pathology and Toxicology, University of Zurich, Switzerland; member, editorial boards of Molecular Pharmacology, Chemico-Biological Interactions, Xenobiotica, Cancer Research, Journal of Toxicology and Environmental Health, and Associate Editor, Pharmacological Reviews. Chairman, Gordon Conference on Drug Metabolism, 1978; Chairman, Drug Metabolism Division, ASPET; Liaison member for ASPET on Scientific Committee on Problems of the Environment of the International Council of Scientific Unions; invited participant, Session Chairman and Co-Editor of Proceedings of International Conference on In Vivo Aspects of Biotransformation and Toxicity of Industrial and Environmental Xenobiotics, Prague, Czech.; guest lecturer, School of Nursing, UNC-CH.

Dr. J. R. Bend: Member, Editorial Advisory Board for Drug Metabolism and Disposition; Visiting Scientist -- C. V. Whitney Marine Laboratory, University of Florida, St. Augustine; Trustee, Mount Desert Island Biological Laboratory,

Salsbury Cove, Maine; member, Committee on Environmental Pharmacology, American Society for Pharmacology and Experimental Therapeutics; external reviewer, National Oceanic and Atmospheric Administration (Dept. of Commerce) Outer Continental Shelf Environmental Assessment Program; member, Scientific Committee and invited participant, "Conjugation Reactions in Drug Biotransformation," Satellite Symposium of the 7th International Congress of Pharmacology, Turku, Finland; invited participant, International Conference on In Vivo Aspects of Biotransformation and Toxicity of Industrial and Environmental Xenobiotics, Prague, Czechslovakia; Associate Managing Editor (U.S.A.) for Chemico-Biological Interactions; Associate Editor, Reviews in Biochemical Toxicology; Session Co-Chairman and invited speaker, Gordon Research Conference on Drug Metabolism (1978).

Dr. R. M. Philpot: Adjunct Assistant Professor, Department of Entomology, North Carolina State University, Raleigh; member of NIEHS Editorial Board; Associate Managing Editor (U.S.A.) for Chemico-Biological Interactions, associate editor, Reviews in Biochemical Toxicology; invited participant in "Isolated Drug Metabolizing Enzymes," Satellite Symposium of the 7th International Congress of Pharmacology, Mainz, West Germany; Session Co-Chairman and invited speaker, Gordon Research Conference on Drug Metabolism (1978).

Dr. J. B. Pritchard: Trustee, Mount Desert Island Biological Laboratory, Salsbury Cove, Maine; Visiting Scientist, C. V. Whitney Marine Laboratory, University of Florida, St. Augustine; Adjunct Associate Professor, Dept. of Pharmacology, University of Florida, Gainesville.

Dr. M. O. James: Adjunct Assistant Professor, Dept. of Pharmacology, University of Florida, Gainesville

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80001-06 LP												
PERIOD COVERED October 1, 1977 to September 30, 1978														
TITLE OF PROJECT (80 characters or less) Microsomal Mixed-Function Oxidase Systems: Specificity and Function														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT														
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">Richard M. Philpot</td> <td style="width: 20%;">Research Chemist</td> <td style="width: 30%;">LP NIEHS</td> </tr> <tr> <td>Other:</td> <td>Cosette S. Shull</td> <td>Chemist</td> <td>LP NIEHS</td> </tr> <tr> <td></td> <td>Charles R. Wolf</td> <td>Visiting Fellow</td> <td>LP NIEHS</td> </tr> </table>			PI:	Richard M. Philpot	Research Chemist	LP NIEHS	Other:	Cosette S. Shull	Chemist	LP NIEHS		Charles R. Wolf	Visiting Fellow	LP NIEHS
PI:	Richard M. Philpot	Research Chemist	LP NIEHS											
Other:	Cosette S. Shull	Chemist	LP NIEHS											
	Charles R. Wolf	Visiting Fellow	LP NIEHS											
COOPERATING UNITS (if any)														
LAB/BRANCH Laboratory of Pharmacology														
SECTION Toxication-Detoxication														
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709														
TOTAL MANYEARS: 4.5	PROFESSIONAL: 3	OTHER: 1.5												
CHECK APPROPRIATE BOX(ES)														
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER														
☐ (a1) MINORS ☐ (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline key														
The objective of this research project is to assess the factors responsible for differences in the substrate specificities among <u>cytochrome P-450-dependent microsomal mixed-function oxidase systems (MFO)</u> from various sources. Present work involves the purification of <u>cytochrome P-450</u>, <u>NADPH-cytochrome c reductase</u>, and lipid fractions from rabbit pulmonary and hepatic microsomes. The <u>cytochrome P-450s</u> are being examined by <u>UV-visible spectroscopy</u>, <u>electron paramagnetic resonance spectroscopy</u>, <u>SDS-gel electrophoresis</u>, and by activities in <u>reconstituted systems</u>. Structural and immunological properties of the cytochromes are also being investigated. The long-range objective of this work is to determine the influence of: 1) multiple forms of the enzymic components of the MFO system; 2) endogenous compounds; and 3) exogenous compounds (inhibitors and inducers) on the substrate specificities of MFO systems from different tissues and species.														

PROJECT DESCRIPTION

METHODS EMPLOYED: Cytochrome P-450 is purified from rabbit pulmonary and hepatic microsomes by procedures developed in this laboratory. The purification of other enzymes and the preparation of phospholipids is done according to existing methods from the literature.

MAJOR FINDINGS AND PROPOSED COURSE:

1) The Rabbit Pulmonary MFO System. Two forms of pulmonary cytochrome P-450 have been isolated and purified. Form I (molecular weight 51,000) can be purified to homogeneity (17-20 nmol cytochrome/mg protein) by a procedure which includes hydrophobic chromatography on phenyl-Sepharose. The substrate specificity, immunological properties, and UV-visible spectra of form I indicate that it is similar to the form of hepatic cytochrome P-450 which is induced by phenobarbital. Pulmonary cytochrome P-450 II can be purified to 8-10 nmol/mg protein using hydrophobic chromatography on octyl-Sepharose. Forms I and II differ with respect to molecular weight, immunological identity, spectra, and substrate specificity. Of particular importance is the finding that the pulmonary cytochromes differ in their abilities to catalyze the metabolism of benzo(a)pyrene, a pulmonary carcinogen, at various positions on the molecule. Form II metabolizes benzo(a)pyrene quite efficiently at the 9,10 positions while form I does not. This difference appears to explain why metabolites produced by form II covalently bind to DNA while those produced by form I do not.

2) The Rabbit Hepatic MFO System. The phenobarbital-induced form of rabbit hepatic cytochrome P-450 has been purified using the methods developed for purification of the pulmonary cytochromes. This hepatic cytochrome cross-reacts with the antibody to the pulmonary cytochrome P-450 I. The hepatic and pulmonary cytochromes also have similar substrate specificities and subunit molecular weights.

The present work involving immunological and structural studies of pulmonary and hepatic forms of cytochrome P-450 will be continued. Localization of the two forms of pulmonary cytochrome P-450 in lung tissue will be attempted using labeled antibodies. Comparison of pulmonary P-450 I and the phenobarbital-induced hepatic P-450 will be extended to detailed immunological and structural studies.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A full understanding of MFO systems is required if drug interaction, drug metabolism, disposition of ingested or inhaled environment pollutants, and pesticide detoxification are to be fully comprehended.

PUBLICATIONS

Arinc, E. and Philpot, R. M.: Catalytic hydroxylation activities and some other properties of partially purified rabbit pulmonary cytochrome P-450. In Ullrich, V., Hildebrandt, A., Roots, I., and Estabrook, R. W. (Ed.): Microsomes

and Drug Oxidations. Oxford, England, Pergamon, 1977, pp. 152-159.

Philpot, R. M. and Arinc, E.: Solubilization, separation, and partial purification of cytochrome P-450 and cytochrome b₅ from hepatic microsomes of the little skate, Raja erinacea. Bull. Mt. Desert Island Biol. Lab 15: 62-64, 1977.

Philpot, R. M., Anderson, M. W., and Eling, T. E.: Uptake, Disposition, and Metabolism of Chemicals by the Lung. In Lenfant, C. (Ed.): Lung Biology in Health and Disease, Vol. IV. Metabolic Function of the Lung (Vane, J. R. and Bakhle, Y. S., ed.) New York, Marcel Dekker, 1977, pp. 123-171.

Wolf, C. R., Szutowski, M., Ball, L., and Philpot, R. M.: The rabbit pulmonary monooxygenase system: Characteristics and activities of two forms of pulmonary cytochrome P-450. Chem.-Biol. Interact. In press.

PROJECT DESCRIPTION

METHODS EMPLOYED: Various enzyme preparations, including trypsin and pronase, have been used for dispersal of lung cells. Populations of cells are separated from the cell digest according to their rates of sedimentation by the technique of centrifugal elutriation. Other methods employed for cell separation include density gradients, differential attachment to tissue culture plates, and affinity chromatography. Cells are studied using light microscopic (including fluorescence microscopy) and electron microscopic techniques. Spectrophotometric, fluorometric, and radiometric methods are used to study cytochrome P-450 and to quantify metabolites of substrates added to cell suspensions.

MAJOR FINDINGS AND PROPOSED COURSE: Techniques are being developed to disperse and separate the many lung cell types in order to localize and study drug metabolism in individual cell populations. Some success has been achieved using pronase digestion followed by cell separation in the elutriator centrifuge and further separation on density gradients. Alveolar type II cells have been enriched to 70% in one cell fraction. This fraction is enriched also in 7-ethoxycoumarin deethylase activity but almost lacking in coumarin hydroxylase activity. Both 7-ethoxycoumarin deethylase and coumarin hydroxylase activities were enriched in another cell fraction which contains the Clara cells and other large cells of the lung. Efforts are now being made to prove whether different cytochromes exist in separate lung cell populations. Immunological studies with cell fractions and antibodies to the purified cytochromes are being used to localize the cytochromes. We are also studying the benzo(a)pyrene metabolite profiles of the various cell fractions since different cytochromes catalyze the formation of different metabolites. Separation of the cell types is also being improved with new density gradients and other modifications of existing techniques. The technique of affinity chromatography may be used to separate specific populations. Future research will focus on the study of toxication and detoxication mechanisms within isolated cell populations to understand the ways chemical and physical stresses alter these systems in lung.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This research seeks to understand some of the factors that affect xenobiotic metabolism in tissues in contact with the environment. Use of lung cells to study drug metabolism may lead to a better understanding of lung damage and repair mechanisms. Comparisons will be made of cellular versus microsomal, purified, and isolated perfused lung xenobiotic metabolism systems to see where differences exist and what this may contribute to understanding toxication and detoxication mechanisms in the body. Studies of changes caused by steroids, metal ions, or other chemicals, or physiological changes (age) on these cellular systems may aid our understanding of when the body is more susceptible to toxic agents and, on a cellular basis, how the body handles these insults. Studies of xenobiotic metabolism in lung cell populations may give us a better understanding of the balance between toxication and detoxication mechanisms and the varied ways chemicals and physiological stresses can alter these systems and this balance.

PUBLICATIONS

Devereux, T. R. and Fouts, J. R.: The effect of dexamethasone treatment on N,N-dimethylaniline demethylation and N-oxidation in pulmonary microsomes from pregnant and fetal rabbits. Biochem. Pharmacol. In press.

Devereux, T. R., Philpot, R. M., and Fouts, J. R.: The effect of Hg^{+2} on rabbit hepatic and pulmonary solubilized, partially purified N,N-dimethylaniline N-oxidases. Chem.-Biol. Interact. 18: 277-287, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80003-05 LP								
PERIOD COVERED October 1, 1977 to September 30, 1978										
TITLE OF PROJECT (80 characters or less) Xenobiotic-Metabolizing Enzyme Activity in Skin and Its Response to Environmental Agents										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT										
<table border="0"> <tr> <td>PI:</td> <td>Roberta J. Pohl</td> <td>Research Biologist</td> <td>LP NIEHS</td> </tr> <tr> <td></td> <td>James R. Fouts</td> <td>Chief</td> <td>LP NIEHS</td> </tr> </table>			PI:	Roberta J. Pohl	Research Biologist	LP NIEHS		James R. Fouts	Chief	LP NIEHS
PI:	Roberta J. Pohl	Research Biologist	LP NIEHS							
	James R. Fouts	Chief	LP NIEHS							
COOPERATING UNITS (if any) Biometry Branch										
LAB/BRANCH Laboratory of Pharmacology										
SECTION Toxication-Detoxication										
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709										
TOTAL MANYEARS: 1.4	PROFESSIONAL: 0.9	OTHER: 0.5								
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS										
SUMMARY OF WORK (200 words or less - underline key words) The project is designed to elucidate the role of <u>xenobiotic-metabolizing enzymes</u> in skin as mediators of toxicity of environmental agents. <u>Mixed-function oxidase</u> (including <u>aryl hydrocarbon hydroxylase</u>), <u>glutathione S-transferase</u> and <u>UDP-glucuronyltransferase</u> activities are measured in pieces of whole skin or epidermis, or in <u>microsomes</u> or <u>cytosol</u> from skin of <u>hairless mice</u> (Hrs/J) after exposure of the mice to <u>ultraviolet radiation</u> , <u>polycyclic hydrocarbons</u> , <u>chlorinated hydrocarbons</u> , etc. The wavelength of maximum absorbance in the carbon monoxide spectra of reduced <u>cytochrome P-450</u> is identified after separating the cytochrome from contaminating mitochondrial pigments.										

PROJECT DESCRIPTION

METHODS EMPLOYED: Whole skin is chopped in 1 mm pieces with a McElwain tissue chopper and aliquots are weighed for assay of mixed-function oxidase activity. In some experiments whole skin or epidermis is disrupted with a Polytron cell disruptor and filtered to obtain homogenate or standard homogenization and ultracentrifuge techniques are used to isolate microsomes and cytosol from the homogenate. Homogenization of chopped skin in a Parr nitrogen decompression cell disruption bomb was tested. Microsomes isolated from this homogenate were contaminated with mitochondrial pigments and could not be used to measure cytochrome P-450 content. Assays for fluorescent metabolites of 7-ethoxycoumarin and 7-ethoxyresorufin were modified to increase sensitivity and reproducibility.

MAJOR FINDINGS AND PROPOSED COURSE: Studies were continued of the effects of chlorinated hydrocarbons, specifically chlordane and Aroclor 1260, and ultraviolet radiation on 7-ethoxycoumarin deethylase (7-EC), 7-ethoxyresorufin deethylase (7-ERF), and UDP-glucuronyltransferase activities in skin from hairless mice (Hrs/J). UDP-glucuronyltransferase (aglycone = 4-methylumbelliferone) was active in freeze-activated pieces of skin when the system was supplemented with UDPGA. No umbelliferone- or resorufin-glucuronide conjugates were detectable in incubation mixtures assayed for 7-EC or 7-ERF activity in skin pieces from chlordane- or Aroclor-treated or untreated mice. UDPGA content in skin from freshly killed mice was 60% of the content in liver per gram tissue. UDPGA disappeared from skin more rapidly than from liver, however.

DMSO and acetone in the quantity used as solvent for topical treatment of mice with chlordane or Aroclor 1260 inhibited 7-EC activity when applied in vitro to skin pieces and for several hours after application in vivo; 1 to 4 days after topical application of DMSO and acetone increases in 7-EC activity were sometimes seen.

No metabolism of p-nitroanisole was detected in mouse skin. Only substrates for AHH (P-448) have been shown, so far, to be metabolized by mouse skin. In vitro inhibition studies with α -naphthoflavone will be used to further define the mixed-function oxidase enzyme form(s) present and/or inducible in mouse skin.

Seasonal and circadian variations in enzyme activity appear to affect mixed-function oxidase activity in mouse skin so that day-to-day variation in control data is marked. These effects will be studied.

Topical treatment of female athymic nude mice with chlordane or Aroclor 1260 had effects on 7-EC and 7-ERF activities in skin similar to those produced in hairless mouse skin. Data collected from this species is compromised, however, by a high incidence of various infections in these mice (resulting from this immune deficiency).

Effects of chlorinated hydrocarbon and UV radiation on xenobiotic-metabolizing enzyme activity in skin will be investigated further. Effects of steroids, bacteriostats, such as the halogenated salicylanilides, organic solvents, etc., on xenobiotic-metabolizing activity in skin will also be studied.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Persistent environmental pollutants, specifically chlorinated organic compounds, are often accumulated in skin. Many other biologically active chemicals are applied to the skin in medicaments, cosmetics, cleaning compounds, etc. Xenobiotic-metabolizing enzymes in the skin may have a role in the locally or systemically expressed toxicity of these compounds. Increased understanding of that role may lead to development of better systems to assess toxicity of chemicals. Manipulation of xenobiotic metabolism may be used to maximize the beneficial effects of chemicals applied to the skin while minimizing toxic reactions.

WITH THE SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80004-06 LP
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Study of Various Factors Affecting Species and Tissue Specific Toxication-Detoxification of Foreign Chemicals.		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Other:	Rajendra S. Chhabra Cristobal L. Miranda	Senior Staff Fellow Visiting Fellow LP NIEHS LP NIEHS
COOPERATING UNITS (if any) Laboratory of Environmental Mutagenesis; Biometry Branch, Comparative Medicine Branch		
LAB/BRANCH Laboratory of Pharmacology		
SECTION Toxication-Detoxification		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 0.75	OTHER: 0.25
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (XX) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline <		
To determine if there were differences in inducibility between hepatic and intestinal microsomal xenobiotic-metabolizing enzymes. <u>Phenobarbital (PB)</u> or <u>3-methylcholanthrene (3-MC)</u> was given orally (po) or intraperitoneally (ip) to mouse, rat, guinea pig, and rabbit. Differences in the inducibility of xenobiotic-metabolizing enzymes due to route of administration if PB were observed in guinea pig, mouse, and rat small intestine but not in liver. Differential effects of po- and ip-administered 3-MC on <u>hepatic aryl hydrocarbon hydroxylase (AHH)</u> and cytochrome P-450 were noted in rat and guinea pig. The induction of intestinal xenobiotic-metabolizing enzymes varied with the animal species and the substrate used. None of the rabbit intestinal xenobiotic-metabolizing enzymes was induced by PB or 3-MC treatment -- even in rabbits fed semi-purified diet. However, rabbit intestinal xenobiotic-metabolizing enzymes were not totally resistant to environmental insult since starvation significantly reduced activities. <u>Vitamin A deficiency</u> or excess feed elicited quantitative and qualitative differences between the species and tissues in their ability to activate or inactivate foreign chemicals.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Microsomal fractions were isolated by differential centrifugation. Spectrophotometric and spectrofluorometric techniques were used for assay of xenobiotic-metabolizing enzyme activities.

MAJOR FINDINGS AND PROPOSED COURSE: Nutritional studies with animals had differential effects on the activation-inactivation of foreign chemicals by liver, small intestine and lung enzyme systems. In vitamin A deficient diet fed animals, the intestinal microsomal enzymes had higher activities than in controls but in liver the activities were lower than in the corresponding controls. The pulmonary microsomal enzymes were unaffected. The epoxide hydrase and glutathione transferase activities in vitamin A deficient animals were similar to controls. Rabbits on a diet containing excess vitamin A (250 IU/gm of diet vs. 15 IU/gm in control diet) for six weeks showed decreased mixed-function oxidase activities (MFO) in lung and gut but without any effect on liver drug metabolizing enzymes.

The studies of various factors affecting extrahepatic metabolism of foreign chemicals will continue to be investigated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Species and tissue differences in the metabolism and response to pollutants by the xenobiotic-metabolizing system may explain both species and strain specific toxicities of pollutants and interactions of pollutants with drugs and chemicals.

PUBLICATIONS

Chhabra, R. S. and Tredger, J. M.: Interaction between drugs and endoplasmic reticulum of intestinal mucosa. In Hathcock and Coon (Ed.): Nutrition and Drug Interactions. New York, Academic Press. In press.

PROJECT DESCRIPTION

OBJECTIVES: 1) To determine how season, sex, and body weight affect hepatic xenobiotic oxidative metabolism, cytochrome P-450 content, and epoxide-metabolizing enzymes in several marine species.

2) To develop a method for determining whether individual teleost and elasmobranch fish have been exposed to inducing agents by assaying hepatic benzo(a)pyrene hydroxylase (AHH) activity.

3) To study how different classes of chemicals, which act as inducers of hepatic xenobiotic metabolism in mammals, affect hepatic xenobiotic metabolism in the sheepshead.

4) To further investigate xenobiotic-metabolizing pathways in the spiny lobster and lobster.

5) To measure taurine N-acyltransferase activity in several marine species and to characterize the enzyme in a suitable aquatic species.

6) To characterize the hepatic mixed-function oxidase system and glutathione S-transferases of representative species from Maine and Florida, before and after pretreatment with polycyclic hydrocarbon-like inducing agents.

7) To identify components of the mixed-function oxidase system in hepatic microsomes from the little skate that are responsible for increased heat lability of this system as compared to that in hepatic microsomes of mammals or Florida marine species.

METHODS EMPLOYED: Published methods were used for preparations of subcellular fractions and for enzyme assays. Fish which were pretreated with chemicals were weighed, labeled with numbered plastic tags through the flesh, and injected with graded doses of chemical or solvent vehicle.

MAJOR FINDINGS AND PROPOSED COURSE: 1) Hepatic epoxide hydrazase activity appears to be season and sex dependent in sheepshead, blue crabs, and stingray. During and after the spawning season (between February and August), hepatic epoxide hydrazase activity in female sheepshead was about 60% of that found in males, whereas the rest of the year the sexes had similar activities. Hepatopancreas microsomes from blue crabs with eggs had less than half the epoxide hydrazase activity of males, while females without eggs had variable activities. Gonadal epoxide hydrazase and glutathione S-transferase activities were about 1/10 and 1/4 of the hepatic activities, respectively, in several species. No sex difference was apparent for mixed-function oxidase activities or glutathione S-transferase activities, although there was a tendency for female sheepshead to have lower cytochrome P-450 content than males. Body weight did not appear to affect the enzymes studied in sheepshead, all of which were at least 350 g.

2) In vitro addition of 7,8-benzoflavone (α -NF) to one set of incubation tubes when assaying AHH activity enabled us to discriminate between true "control" fish, and fish in which hepatic AHH activity was apparently "induced." α -NF stimulated AHH activity of hepatic microsomes from controls about 5-fold, and inhibited AHH activity about 3-fold in hepatic microsomes from sheephead that were maximally induced by treatment with 3-methylcholanthrene (3-MC). The effect on activity in hepatic microsomes from sheephead that had been treated with 3-MC, but were not maximally induced, showed a spectrum of effects from slight stimulation through no effect to slight inhibition. During 1977 we found 14 out of 94 sheephead caught from the wild and maintained at Whitney Marine Lab had elevated AHH activity which was inhibited by α -NF. AHH activity in sheephead which had not to our knowledge been exposed to chemicals, and which were not induced according to the α -NF criterion, nevertheless showed a wide variation in AHH activity between 0.47 and 5.0 F.U./min/mg protein. Thus, the α -NF test provides a useful means of determining whether untreated fish had previously been exposed to inducing agents.

Similarly, 11 of 13 winter flounder assayed in Maine were found to have hepatic AHH activities that were inhibited by 10^{-4} M α -NF (in vitro). 7-Ethoxyresorufin deethylase (7-ERF) activities were also higher in hepatic microsomes from the fish with higher AHH activities (which were inhibited by α -NF). Collectively, this data demonstrates that the hepatic mixed-function oxidase system of these flounder was partially induced. Whether or not this induction was due to exposure to polycyclic hydrocarbon-like compounds in the field is still unresolved.

3) We have shown that pretreatment of sheephead with 3-MC induced AHH and 7-ethoxycoumarin O-deethylase (7-EC) activities in hepatic microsomes by 4 to 8 days after injections of a dose of 10 mg/kg, depending on time of year. We have not achieved a similar effect with the less carcinogenic type 2 inducers, dibenz(a,h)anthracene (DBA) and 5,6-benzoflavone (β -NF). In some experiments with all three inducers, cytochrome P-450 contents were also increased, but the difference between treated and control fish was not always statistically significant, and we did not observe a spectral shift in the peak to 448 nm which is reported in mammals. None of these agents induced benphetamine N-demethylase or epoxide hydrase activities in hepatic microsomes or glutathione S-transferase activity in hepatic cytosol fractions. Hepatic AHH and 7-EC activities were induced in flounder that were treated with 3-MC. Other ubiquitous environmental contaminants, the polychlorinated biphenyls (PCBs), induce several pathways of xenobiotic metabolism in mammals, as do the related polybrominated biphenyls (PBBs). We found that administration to sheephead of a mixture of PCBs, Arochlor 1254, or a mixture of PBBs, Firemaster FFI, caused a more general increase in xenobiotic-metabolizing activities than did 3-MC, DBA, or β -NF. Doses of 50 to 100 mg/kg of Arochlor 1254 or 15 mg/kg of the more toxic Firemaster FFI induced AHH and 7-EC activities 10-fold and 5-fold, respectively, and doubled cytochrome P-450 contents of livers. Maximum induction was attained 1 month after injection. In most experiments we found a 1.5- to 2-fold

increase in benzphetamine N-demethylase activity of treated fish. The effect on epoxide hydrase activity could not be clearly interpreted due to the sex difference in activity at the time of year the experiments were performed. Attempts to induce hepatic xenobiotic-metabolizing enzymes of sheephead with the type 1 inducing agent, phenylbutazone, have not succeeded. Multiple injections of phenylbutazone or exposure to 100 or 200 ppb in the seawater caused no reproducible change in activity of AHH, 7-EC, benzphetamine N-demethylase, epoxide hydrase, or glutathione S-transferase, or in cytochrome P-450 content.

4) We have previously shown that the low activities of oxidative enzymes found in hepatopancreas microsomes from the spiny lobster are not due to lack of cytochrome P-450 or to the ability to bind substrates to the cytochrome. It now appears that although low activities of NADPH-cytochrome c reductase are present in hepatopancreas microsomes from the spiny lobster, these microsomes have no NADPH-cytochrome P-450 reductase activity. Addition of lobster hepatopancreas to sheephead hepatic microsomes steadily abolished the cytochrome c or cytochrome P-450 reductase activities of sheephead hepatic microsomes. Other spectral studies suggest that carbon monoxide, or a ligand with properties similar to carbon monoxide, is present in lobster hepatopancreas microsomes, and this may inhibit oxidative metabolism.

5) Using ¹⁴C-phenylacetylcoenzyme A as the acyl donor, we found taurine N-acyltransferase activity in hepatic, renal, and gonadal mitochondria from a number of teleost and elasmobranch fish. Stingray kidney was chosen as a source to further characterize this enzyme. By comparison of the properties of taurine N-acyltransferase with glutamate dehydrogenase and succinate dehydrogenase, we determined that taurine N-acyltransferase, like the related mammalian enzyme glycine N-acyltransferase, is located in the matrix of the mitochondria. A soluble preparation of enzyme was obtained by sonicating stingray renal mitochondria and centrifuging at 15,500 g. Activity remained in the supernatant. Taurine- and glycine-N-acyltransferase shared a number of characteristics in the soluble preparations, such as pH optimum and apparent Km's for phenylacetylCo A.

Mitochondria prepared from hepatopancreas or green gland of the spiny lobster or blue crab had very low taurine N-acyltransferase activities. This was not unexpected since phenylacetic acid is not excreted as a taurine conjugate by these species.

6. 7-ERF and 7-EC activities in hepatic microsomes from little skate are more labile to heat than are those in hepatic microsomes from male New Zealand White rabbits. Fatty acids in both simple lipids and phospholipids extracted from skate hepatic microsomes were less saturated than were those extracted from rabbit hepatic microsomes. 7-ERF activity was more heat labile in rabbit hepatic butanol:acetone extracted-microsomes reassociated with lipid extracted from skate hepatic microsomes than in those reassociated with lipid extracted from rabbit hepatic microsomes.

7) The glutathione S-transferases of little skate liver cytosol have been separated and partially purified. Transferase activity was eluted from a DEAE-cellulose column with a KCl gradient. Four peaks of glutathione S-transferase activity were obtained using 1,2-dinitro-4-chlorobenzene as substrate. All peaks were also active with benzo(a)pyrene 4,5-oxide. Non-SDS polyacrylamide gel electrophoresis of each of the activity peaks demonstrated they each had a different migratory pattern.

8) The metabolites obtained upon incubation of benzo(a)pyrene with hepatic microsomes of DBA-pretreated little skates were resolved by HPLC. The major metabolites were 3-hydroxybenzo(a)pyrene ($\approx 40\%$ of total metabolites), diones ($\approx 20\%$), and 9-hydroxybenzo(a)pyrene ($\approx 20\%$). Smaller amounts of benzo(a)pyrene 4,5-dihydrodiol and benzo(a)pyrene 9,10-dihydrodiol were also detected.

The proposed course is as follows: 1) In collaboration with Dr. P. Cardeilhac, we plan to determine if the apparent effect on epoxide hydrolase activity during spawning can be duplicated at other times of the year by administration of hormones which induce spawning. Gonadal epoxide-metabolizing enzymes will also be studied.

2) Hepatic AHH activity in control fish from Maine and Florida will be monitored at regular intervals to see if there is any pattern in the apparent induction of control fish. In this connection, samples of liver will be analyzed for hydrocarbons and chlorinated pesticides, in collaboration with members of the Chemistry Branch at NIEHS. The effect of administering inducing agents on AHH activity in some extrahepatic organs (gills and gonads) will be investigated.

3) In vivo metabolism of selected pollutants will be studied in control versus induced fish, using conditions established for enzyme induction in vitro.

4) Further investigation of the xenobiotic-metabolizing system of crustacea will be carried out. We plan to isolate cytochrome P-450 from lobster microsomes, reconstitute these with reductase and lipid fractions from rat or sheephead liver, and determine whether xenobiotic oxidation can then be supported.

5) Using the solubilized preparation of taurine N-acyltransferase from stingray renal mitochondria, we plan to further purify transferase activity and compare this with glycine transferase activity.

6) Arrhenius plots of 7-ERF activity in lipid extracted and reassociated microsomal preparations will be analyzed to identify possible effects of lipid-phase changes on the rate of biotransformation of chemicals at temperatures of physiological importance to poikilothermic animals.

7) The glutathione S-transferases from little skate liver will be further purified for comparison to mammalian glutathione transferases using gel electrophoresis, isoelectric focusing, immunological, and amino acid analysis procedures as well as catalytic activity towards a number of electrophilic substrates including alkene and arene oxides.

8) The two forms of cytochrome P-450 from DBA-pretreated little skate hepatic microsomes will be further purified and characterized by comparison to the cytochrome of untreated little skates as well as by comparison to characterized mammalian cytochromes available in our laboratory.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The marine ecosystem is contaminated with substantial amounts of pesticides, industrial by-products, and other synthetic organic chemicals which are produced in huge quantities by our technologically oriented society. The ability or inability of marine species, particularly those that are edible or those which are important in the aquatic food chain, to biotransform and excrete these xenobiotics is relevant both to the subsequent fate of these species (in the face of increasing pollution) and to their potential value and hazard as direct and indirect foodstuffs for man. Comparison of the enzymes involved in metabolizing xenobiotics in different species should lead to a better mechanistic understanding of the overall effects of xenobiotics on the animal, and of animals on xenobiotics.

Moreover, a detailed understanding of metabolic activation and deactivation pathways in marine animals may allow us to predict those species most likely to be affected by carcinogens, mutagens, teratogens, or cytotoxins in the environment, and allow us to use them as sentinel or early warning indicators of toxic environmental contaminants.

PUBLICATIONS

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Philpot, R. M., James, M. O., and Bend, J. R.: Metabolism of Benzo(a)pyrene and Other Xenobiotics by Microsomal Mixed-Function Oxidases in Marine Species. Sources, Effects, and Sinks of Petroleum in the Aquatic Environment. American Institute of Biological Sciences Symposium, Washington, D. C., August, 1976, pp. 184-199.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80006-07 LP
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Environmental Contaminants: Uptake, Distribution, Metabolism, Excretion, and Storage Sites in Marine Species		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Other:	John R. Bend Margaret O. James John B. Pritchard Robert P. Weatherby Elizabeth R. Bowen Gary R. Foureman Anthony M. Guarino James R. Fouts	Visiting Scientist Senior Staff Fellow Research Physiologist Visiting Fellow Bio Lab Technician Biologist Research Pharmacologist Chief LP NIEHS LP NIEHS LP NIEHS LP NIEHS LP NIEHS LP NIEHS LT NCI LP NIEHS
COOPERATING UNITS (if any)		
Laboratory of Toxicology, National Cancer Institute; Biometry Branch		
LAB/BRANCH Laboratory of Pharmacology		
SECTION Marine Pharmacology and Biomedicine		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 1.7	PROFESSIONAL: 0.9	OTHER: 0.8
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline key words) The long-range objectives of this project are to study the <u>in vivo uptake</u> , <u>distribution</u> , <u>metabolism</u> , and <u>excretion</u> of single, purified radiolabeled <u>environmental contaminants</u> , such as <u>2,4,5-trichlorophenoxyacetic acid</u> , <u>polychlorinated biphenyl isomers</u> , and <u>hydrocarbons</u> , in <u>vertebrate and invertebrate marine species</u> that serve as human food sources. The role of <u>environmental temperature</u> and <u>exposure to other pollutants</u> on the processes involved are also investigated. Particular attention is focused upon potential <u>carcinogens</u> , <u>mutagens</u> , <u>teratogens</u> , and <u>cytotoxins</u> that may occur as food residues due to exposure of aquatic animals.		

PROJECT DESCRIPTION

METHODS EMPLOYED: At various times after administration (generally parenteral) of radiolabeled compounds, specimens are sacrificed, plasma and tissues are removed, and aliquots are oxidized and/or solubilized prior to liquid scintillation counting. Urinary and biliary excretion rates are also monitored where appropriate. Metabolites are identified by standard procedures. We have now established a procedure for collecting urine from the southern flounder, at the Whitney Lab, by means of an indwelling catheter.

MAJOR FINDINGS AND PROPOSED COURSE: 1) ^{14}C -Phenylacetic acid was excreted in urine by the southern flounder as a taurine conjugate. The overall half-life was about 8 hr, and over 95% of the excreted ^{14}C was present as the taurine conjugate. ^{14}C -DDA [bis(4-chlorophenyl)acetic acid, a metabolite of DDT] was also excreted as a polar metabolite in urine which was isolated and identified as the taurine conjugate. The overall half-life of DDA in the southern flounder was about 20 hr. Less than 1% of an intramuscular dose of ^{14}C -hexadecane was excreted in urine by 24 hr after injection. At this time, ^{14}C was concentrated in the liver.

2) Most of a dose of ^{14}C -phenylacetic acid was taken up from circulating hemolymph into hepatopancreas by the spiny lobster and excreted slowly in feces as a polar metabolite. The half-life of elimination of the metabolite was about 2 weeks. The metabolite had a M.W. of less than 1000, and was a conjugate with one or more amino acids, but the amino acid(s) component has not yet been established. The related carboxylic acid herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) was eliminated very quickly from lobster after injection into the circulatory system. The organ of excretion seemed to be the green gland, and the half-life of elimination was less than 6 hr. 2,4-D was not metabolized by the lobster.

3) ^{14}C -Hexadecane and ^{14}C -octane were mainly taken up into hepatopancreas of the spiny lobster, after injection into the pericardial sinus, and were excreted slowly, probably via the intestines. The overall half-life of elimination of octane was about 1 week, and of hexadecane about 2 weeks, but the rate of elimination from certain compartments, e.g., tail muscle and gonads, was often slower. Thus 3% of a dose of hexadecane was present in tail muscle 8 weeks after injection and 1% of a dose of octane was found in tail muscle 4 weeks after dosing. Less than 1% of the dose was present in gonads one day after injection of either hydrocarbon, but this small amount persisted up to the longest time assayed for each (4 weeks for octane, 8 weeks for hexadecane). The rate of elimination of both hydrocarbons was slower in the winter than in summer.

4) ^{14}C -Octane was also primarily distributed into the hepatopancreas of the Maine lobster following pericardial injection, and it was excreted substantially more slowly than by the spiny lobster. In fact, 20% of the dosed radioactivity was retained in the lobster 15 days after administration, primarily in the hepatopancreas.

The proposed course is as follows: 1) The metabolite of phenylacetic acid, which was found in lobster hepatopancreas, will be identified by conventional techniques, and a knowledge of the identity of this metabolite used to study the conjugation in vitro of phenylacetic acid.

2) Studies will be continued on the uptake, metabolism, and elimination of selected radiolabeled aliphatic and aromatic constituents of crude oil, using at least one vertebrate and invertebrate species. The effect of pre-exposure of experimental animals to foreign organic chemicals, such as PCBs, chlorinated insecticides and hydrocarbons, and to heavy metals on the subsequent uptake, metabolism and elimination of these aliphatic and aromatic radiolabeled crude oil constituents will also be studied in Maine and Florida marine animals. Other routes of administration of chemicals will be investigated.

3) Using xenobiotic conjugates which are excreted in urine and bile, such as ^{14}C -phenacetyl glycine, the effect of environmental temperature on urinary and biliary excretion will be studied in Florida fish that have been acclimated to a variety of temperatures in the laboratory for a prolonged period (2 weeks - 1 month) of time.

4) Pharmacokinetics of selected pollutants in marine species will be compared with similar data obtained in rats (by the Laboratory of Pharmacokinetics, NIEHS) as part of a general program to develop models for pollutant distribution in different species and to be able to extrapolate pollutant distribution data obtained in animals to man.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Marine pollution by chemicals such as crude and refined petroleum products, pesticides, polychlorinated biphenyls, polybrominated biphenyls, and polycyclic aromatic hydrocarbons is a problem of significant importance to human health since many aquatic organisms concentrate such pollutants and man relies upon these species for substantial quantities of food. Carefully controlled pharmacokinetic experiments will identify species that can be sampled in the field to monitor both short and longer term pollutant levels. The study of interaction between environmental contaminants and other xenobiotics, including drugs, food additives, and pesticides, is important since mixtures of such pollutants are always encountered in the environment. Such investigations in marine species, with regard to competition for metabolic and excretion pathways or storage sites, may assist in understanding increased (or decreased) toxicity of pollutant-drug mixtures as they affect higher animals, including man.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80007-07 LP
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Hepatic and Extrahepatic Conjugation and Oxidation Metabolic Pathways for Xenobiotics in Mammals		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Other:	John R. Bend Brian R. Smith Hasan Mukhtar Oscar Hernandez Jennifer Van Anda Louise M. Ball Gary L. Foureman Richard M. Philpot Theodora R. Devereux Insu P. Lee	Visiting Scientist Staff Fellow Visiting Associate Visiting Associate Graduate Student Visiting Fellow Biologist Research Chemist Research Biologist Research Pharmacologist
		LP NIEHS LP NIEHS LP NIEHS EBCB NIEHS LP UNC/CH LP NIEHS LP NIEHS LP NIEHS LP NIEHS LP NIEHS LEM NIEHS
COOPERATING UNITS (if any) Laboratory of Environmental Toxicology; Biometry Branch; Environmental Biology and Chemistry Branch		
LAB/BRANCH Laboratory of Pharmacology		
SECTION Toxication-Detoxication		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 5.8	PROFESSIONAL: 5.0	OTHER: 0.8
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline k Several <u>arene</u> and <u>alkene oxides</u> are known to react covalently with macro- molecules, including nucleic acids, and to transform cells <u>in vitro</u> , suggesting their role as ultimate carcinogens, mutagens, and cytotoxins. We are studying the mixed-function oxidases, which convert unsaturated <u>hydrocarbons</u> to <u>epoxides</u> , and the further <u>metabolism</u> of arene and alkene oxides by soluble fraction <u>glutathione S-transferases</u> and microsomal <u>epoxide hydrase</u> in hepatic and extra- <u>hepatic tissues</u> (including <u>testis</u> , <u>ovary</u> , and <u>adrenal</u>). The relative quantita- <u>tative importance</u> of the two <u>pathways</u> for epoxide metabolism is being studied <u>in</u> <u>vitro</u> in intact <u>pulmonary cells</u> , and in <u>isolated perfused rat liver</u> and <u>rabbit</u> <u>lung preparations</u> .		

PROJECT DESCRIPTION

OBJECTIVES: To characterize in intact tissue the pathways responsible for the metabolic conversion of polynuclear aromatic hydrocarbons to epoxides and for the decomposition of these reactive intermediates; 2) to assess the importance of these enzyme systems in protecting tissues from reactive intermediates; 3) to evaluate the influence of cellular integrity and intracellular location of enzymes on the metabolism of reactive intermediates; 4) to correlate dosage levels with activity of epoxide-metabolizing pathways and toxicity (including histopathology and covalent binding of radioactivity to macromolecules) and rates of excretion in isolated perfused organs; 5) to study developmental patterns of epoxide metabolism in germinal and steroidogenic tissues; and 6) to investigate position-specific hydroxylation of model substrates in reconstituted mixed-function oxidase (MFO) and other in vitro systems.

METHODS EMPLOYED: Differential centrifugation, spectroscopic, fluorometric, and radiochemical assays, organ perfusion procedures, ion exchange, thin-layer, paper, and high-pressure liquid chromatography, and synthetic organic chemistry.

MAJOR FINDINGS AND PROPOSED COURSE: 1) The perinatal development of enzymes responsible for epoxide metabolism was studied in rat adrenal, ovary, and testis. Adrenal P-450 was 0.83 and 0.28 (nmol per mg mitochondrial or microsomal protein, respectively) at day 1 and increased to adult levels by day 40. Epoxide hydrase (EH) activity, about 50% of control activity at day 1, also increased to adult levels by day 40. Ovarian P-450 levels and EH activity developed rapidly between 2 and 3 weeks of age and reached a maximum around day 40.

2) Rat testicular EH activity increased dramatically at the onset of puberty to a maximum value by day 45. Glutathione S-transferase (GS-T) activity was 50% of adult values at day 6, and developed slowly to a maximum value by day 165.

3) Microsomal and soluble fractions prepared from rat spermatogenic cells had about twice the EH and GS-T activities of similar fractions prepared from interstitial cells. On the other hand, benzo(a)pyrene hydroxylase (AHH) activity and cytochrome P-450 content were at least 2-fold greater in microsomes from interstitial cells compared to those from spermatogenic cells.

4) Ovarian GS-T activities were followed during pregnancy. A 112% increase toward styrene oxide (SO) was seen on the 11th day after conception, whereas activities toward other substrates were increased only 33-40% (benzo(a)pyrene 4,5-oxide [BPO]; 1,2-dichloro-4-nitrobenzene; 1-chloro-2,4-dinitrobenzene).

5) Rat sperm was found to contain GS-T and Δ^5 -3-ketosteroid isomerase activities. Sperm GS-T specific activities toward BPO, SO, and 1,2-dichloro-

4-nitrobenzene were 1.9, 0.4, and 1.4%, respectively, of the hepatic GS-T activities. Sperm Δ^5 -3-ketosteroid isomerase activity was 2.46 nmol/min/mg protein, about 4.1% of the hepatic activity.

6) The enzymes responsible for epoxide metabolism were studied in rat liver nuclei and nuclear membranes. MFO and EH specific activities in nuclei were approximately 5-20% of microsomal preparations. AHH and EH activities were 2-5 times greater in nuclear membranes than in intact nuclei. Kinetic investigations indicated that EH in nuclear membranes has a K_m value different from that of the microsomal enzyme. The activities of the MFO enzymes in the nuclear membrane were responsive to the inductive effects of phenobarbital and 3-methylcholanthrene.

7) The administration of trans-stilbene oxide to rats resulted in increased hepatic microsomal and nuclear EH and AHH activities. Hepatic microsomal aminopyrine, benzphetamine, and ethylmorphine N-demethylase activities were also increased. These increases were dose and time dependent. No changes were observed in microsomal or nuclear cytochrome P-450 or NADPH-cytochrome c reductase or cytosolic GS-T activity.

8) A method has been developed by which the enzymatic conjugation of glutathione with BPO can be monitored fluorometrically. Fluorometric scans (ex. 300 nm) of an active incubation system show a time-dependent emission at 371 nm which required the presence of reduced glutathione. The rate of increase in the emission intensity was directly related to the amount of enzyme. This sensitive assay can be used to monitor directly the time-course of BPO with glutathione conjugation.

9) The metabolism of benzo(a)pyrene (BP) by the isolated perfused rabbit lung was investigated using HPLC techniques for qualitative and quantitative analysis of metabolites. In this system, 170 nmol of BP per g wet weight of lung were metabolized in 30 min. The main product was shown to be 3-hydroxybenzo(a)pyrene (60% of total metabolites), with quinones (20%) and BP 9,10-dihydrodiol (10%) being the only others of any importance. Pretreatment of the rabbits with 3-methylcholanthrene did not affect the overall rate of metabolism, but slightly increased the proportion of 9-hydroxybenzo(a)pyrene and BP 9,10-dihydrodiol formed.

10) The metabolism of BP 7,8-dihydrodiol was studied in the isolated perfused rabbit lung. The diol was converted to water-soluble metabolites at a rate of 258 pmol/min/g lung. (No tetrahydrotetrols were detected.) Very small amounts of dihydrodiol-derived radioactivity were covalently bound to lung DNA (2 pmol/mg) or protein (3.3 pmol/mg).

11) The activity of rabbit lung EH and GS-T toward BP was investigated at various levels of organization: intact lung, pulmonary cells, lung homogenates, S-9 fractions, microsomes, and soluble fractions. The intact lung metabolized BP at a rate of 4.2 nmol/min/g lung for the EH pathway, and 6.5 nmol/min/g lung for the transferase pathway. Isolated pulmonary cells

metabolized BP faster than the intact tissue: 13.4 nmol/min/g lung for EH, and 18.7 nmol/min/g lung for GS-T. The intracellular glutathione concentration appeared to have a limiting effect on the rate of the transferase reaction, and this rate could be increased to 62 nmol/min/g lung by sonicating the cells and supplementing the incubation mixtures to 5 mM in glutathione. EH activity was not changed by sonicating the cells, indicating that the cell membrane is readily permeable to BPO.

EH and GS-T activities were also higher in in vitro assays using subcellular fractions compared to activities seen in the intact tissue.

From comparing results from tissue homogenates, S-9 fractions, microsomes, and soluble fractions, it appeared that association of the BP with non-enzymatic protein was responsible for the lower rates of the two epoxide-metabolizing pathways seen in pulmonary cells and especially in the intact tissue. However, uptake from the perfusion medium and tissue and intracellular distribution of BPO by the isolated lung is another factor contributing to reduced rates of metabolism in the intact tissue.

12) The isolated perfused rat liver was used to study the hepatic metabolism and excretion of BPO. BPO was rapidly metabolized by both EH and GS-T. Metabolism occurred at much faster rates than the metabolites could be released into the perfusion medium and excreted in the bile. The dihydrodiol of BPO is further conjugated with glucuronic acid, and to a lesser extent with sulfate. Biliary excretion was the principal route of disposal of conjugated metabolites. These findings were corroborated in whole animal experiments.

13) A chromatographic assay procedure was developed for simultaneous determination of EH and GS-T activities in intact cells and subcellular fractions.

14) The metabolism of S0 by the perfused rat liver was studied at doses of 1, 10, 50, 100, and 500 $\mu\text{mol/liver}$. The rate of metabolism of S0 by EH and by GS-T was dependent on substrate concentration. However, the EH activity at the 1 and 10 μmol doses of S0 was linear with time for only about 7 min after the addition of substrate to the perfusion medium. Increasing the amount of S0 added to the perfusion medium extended the period of high activity of EH. At the 500 μmol dose, the rate of the EH reaction was linear with time for the entire 60-min duration of the experiment. The transferase reaction was active for 60 min in all cases. Increasing the amount of S0 added to the perfusion medium resulted in increased excretion of biliary metabolites except at the 500 μmol dose where bile flow and excretion of biliary metabolites were reduced. At this dose, the extent of covalent binding of S0-derived radioactivity to liver tissue protein, DNA, and RNA was greatly elevated relative to the amount of covalent binding that occurred at lower doses. The 500 μmol dose of S0 caused severe liver damage, which was observed histologically and also measured biochemically as release of hepatic transaminase enzymes into the perfusion medium.

The proposed course is as follows:

- 1) In an attempt to characterize endogenous regulators of drug-metabolizing activity in rat liver, mothers and newborn pups will be treated with compounds such as dexamethasone and thyroxine. The effects of these substances on cytochrome P-450, EH, and GS-T will be studied.
- 2) The activities of drug-metabolizing enzymes will be determined in outer and inner nuclear membranes from rat liver and extrahepatic tissues.
- 3) Glutathione conjugates of BPO will be synthesized. This will serve to standardize the fluorometric GS-T assay and to further confirm the identity of the thioether conjugates formed by metabolism of BPO in the lung and in the liver.
- 4) Glucuronide and sulfate conjugates of BP-4,5-dihydrodiol and styrene glycol will be produced by chemical or biosynthetic means and then characterized. These standards will be used in the studies described below.
- 5) Glucuronyltransferase and sulfotransferase activities of rabbit lung and rat liver will be characterized in vitro using subcellular fractions and BP 4,5- and 7,8-dihydrodiols as substrates.
- 6) The ability of the isolated perfused rabbit lung and rat liver to conjugate BP 4,5- and 7,8-dihydrodiols and styrene glycol with sulfate and glucuronide will be evaluated.
- 7) The metabolism of BP will be examined in the isolated perfused rabbit lung and in rabbit lung subcellular preparations to assess the importance of hydroxylation of different positions on the molecule (using the specific inhibitor α -naphthoflavone) and to evaluate the extent of conjugation with sulfate, glucuronide, and glutathione.
- 8) The metabolism of BP and styrene will be investigated in the isolated perfused liver to determine the effect of the source of epoxides (extracellular administration compared to generation by intracellular metabolism) on the relative importance of EH and GS-T in decomposing reactive intermediates.
- 9) The effect of piperonyl butoxide, a potent MFO inhibitor, on the pulmonary metabolism of BP will be investigated. The effect of piperonyl butoxide on the covalent binding of BP-derived radioactivity to cellular macromolecules will be determined.
- 10) A model substrate, p-methylanisole, will be used to investigate position-specific hydroxylation by various mammalian in vitro systems.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Xenobiotics or their chemically reactive metabolic intermediates (e.g., alkene or arene oxides) are detoxicated by several pathways, principally

involving conjugation in liver, lung, gut, and other extrahepatic tissues, and excreted from the animal. The activities of these conjugation and excretory mechanisms may be important factors in determining the susceptibility of organs, or individuals, to environmental agents. The developmental patterns of conjugation pathways active in detoxication are of importance since these may determine the susceptibility of the fetus and neonate to environmental contaminants and may also be related to chemical carcinogenesis and teratogenesis. In the real-life situation one is very rarely exposed to a single xenobiotic; rather, one comes in contact with a large number of synthetic organic chemicals including food additives, drugs, plasticizers, insecticides, hydrocarbons, etc. For this reason investigations concerning interactions between more than one foreign compound are of relevance to environmental health.

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Smith, B. R. and Bend, J. R.: Metabolism of benzo(a)pyrene 4,5-oxide by isolated perfused rabbit lung. Second International Symposium on Polycyclic Aromatic Hydrocarbons, Columbus, Ohio, September, 1977. In press.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80009-07 LPK								
PERIOD COVERED October 1, 1977 to September 30, 1978										
TITLE OF PROJECT (80 characters or less) Pharmacokinetic and Pharmacodynamic Functions of the Lung										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI:</td> <td style="width: 33%;">Alan G. E. Wilson</td> <td style="width: 33%;">Visiting Associate</td> <td style="width: 15%;">LPK NIEHS</td> </tr> <tr> <td>Other:</td> <td>Ada C. Kung</td> <td>Visiting Fellow</td> <td>LPK NIEHS</td> </tr> </table>			PI:	Alan G. E. Wilson	Visiting Associate	LPK NIEHS	Other:	Ada C. Kung	Visiting Fellow	LPK NIEHS
PI:	Alan G. E. Wilson	Visiting Associate	LPK NIEHS							
Other:	Ada C. Kung	Visiting Fellow	LPK NIEHS							
COOPERATING UNITS (if any) Inhalation Toxicology Section, Environmental Biology and Chemistry Branch										
LAB/BRANCH Laboratory of Pharmacokinetics										
SECTION 										
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709										
TOTAL MANYEARS: 2	PROFESSIONAL: 1	OTHER: 1								
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (X) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS										
SUMMARY OF WORK (200 words or less - underline key The objective of this research project is to investigate the <u>pharmacokinetic functions of the lung</u> with regard to both <u>endogenous and exogenous chemicals</u>. An <u>isolated perfused lung (IPL) system</u> has been developed, which permits examination of the pharmacokinetics of chemicals reaching the lung both from the circulation and from the inhaled air. Pharmacokinetic data will be correlated with toxicological, physiological, and biochemical changes induced during the accumulation of pollutants by the lung. <u>Pharmacokinetic models</u> will be constructed and related to environmentally induced pulmonary damage. The role of the lung in the whole body disposition of environmental agents will be assessed and data will be incorporated into pharmacokinetic models for the uptake and disposition of chemicals by the lung.										

PROJECT DESCRIPTION

PART I. STUDIES ON THE PHARMACOKINETICS OF ACCUMULATION AND METABOLISM OF CHEMICALS AND POLLUTANTS BY THE LUNG.

METHODS EMPLOYED: The lung perfusion apparatus consists of an artificial thorax, a recirculating or "single-pass" perfusate supply, and an air supply. The lungs are ventilated with 95% air-5% CO₂ by applying an alternating negative pressure to the artificial thorax. The circulatory system was designed with a perfusate reservoir directly above the lungs to which the labeled chemicals were added. Perfusate samples are removed and analyzed following passage through the lungs. This apparatus permits studies of the rates of uptake and efflux of chemicals by the lung. The perfused lung has been modified to permit studies of the metabolism and retention of inhaled chemicals. The trachea is connected directly to either a vapor or an aerosol generator. Appearance of the chemical and its metabolites in the perfusate and expired air are continuously monitored. The system is designed to permit study of the effect of exposure to the inhaled chemical on the physiological function of the lung.

Lung slices, incubated in a Krebs-Ringer bicarbonate buffer containing 5 mM glucose are also employed to obtain information on the uptake and metabolism of chemicals by lung tissue.

MAJOR FINDINGS AND PROPOSED COURSE: Considerable effort and time was devoted to adapting the IPL system to permit pharmacokinetic studies of the uptake, distribution, and metabolism of inhaled chemicals. Considerable concern was taken over insuring a correct inhalation system for the IPL and satisfying safety considerations. The system has been developed to permit measurement of changes in pulmonary function due to the inhaled chemicals.

The proposed course is as follows: 1) To further develop the IPL to study the pharmacokinetics of inhaled chemicals.

2) To examine the uptake and metabolism of environmental agents as model chemicals and relate their disposition to the induction of pulmonary damage.

3) To study the effect of modifiers on the uptake and metabolism of inhaled compounds, e.g., particulates.

4) To elucidate the mechanism(s) and differences between the pulmonary metabolism and kinetics of inhaled and blood-borne chemicals.

PART II. STUDIES ON THE EFFECT OF ENVIRONMENTAL AGENTS ON THE PHARMACOKINETICS OF UPTAKE AND METABOLISM OF ENDOGENOUS SUBSTANCES BY THE LUNG.

METHODS EMPLOYED: See Part I.

MAJOR FINDINGS AND PROPOSED COURSE: 5-Hydroxytryptamine (5-HT) is removed from the circulation in the lung by a unidirectional transport system which is sodium dependent. 5-HT is metabolized by intracellular monoamine oxidase (MAO) to 5-hydroxyindole acetic acid and this metabolite rapidly diffuses back into the pulmonary circulation. As part of an investigation of the effect of chemicals on the pulmonary clearance of endogenous compounds, we have studied the effect of exposure to the industrial solvent morpholine on the pulmonary uptake and metabolism of ¹⁴C-5-HT. Exposure of rats to morpholine (LD₅₀) over a 5-week period produced a significant decrease in the pulmonary clearance of 5-HT. This was not due to an effect on the pulmonary transport system for 5-HT, but due to an inhibition of 5-HT metabolism by MAO. This was confirmed by the finding that morpholine is a potent MAO inhibitor, displaying competitive inhibition kinetics for a variety of MAO substrates. The substrate specificity of the pulmonary MAO's (i.e., A and B types) for endogenous amines is presently being investigated.

The proposed course is as follows: Extension of our studies on the effect of exposure to environmental pollutants on the pulmonary pharmacokinetics of endogenous compounds.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

The lung is an important site for the uptake and detoxification of a number of important endogenous compounds. Understanding the significance of this pulmonary accumulation and metabolism is essential to an understanding of lung function and the effect of exposure to environmental pollutants on the lung. Elucidation of the mechanisms of pulmonary uptake and metabolism of chemicals may permit early prediction of pulmonary damage, such as the reduced pulmonary clearance of 5-HT seen in oxygen toxicity. In addition such knowledge may allow possible prediction of toxic interactions between endogenous and exogenous substances.

PUBLICATIONS

Anderson, M. W., Philpot, R. M., Bend, J. R., Wilson, A. G. E., and Eling, T. E.: Pulmonary uptake and metabolism of chemicals by the lung. Excerpta Medica Clin. Toxicol. XVIII: 85-140, 1977.

Wilson, A. G. E. and Kung, H. C.: Effect of morpholine on the pulmonary clearance of 5-hydroxytryptamine. Biochem. Pharmacol. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80031-02 LP			
PERIOD COVERED October 1, 1977 to September 30, 1978					
TITLE OF PROJECT (80 characters or less) Role of Altered Membrane Function in Xenobiotic Toxicity					
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%;"> <tr> <td style="width: 33%;"> PI: John B. Pritchard Galen J. Neufeld </td> <td style="width: 33%;"> Research Physiologist IGPA Fellow </td> <td style="width: 33%;"> LP NIEHS Emporia St. Univ., Kan. </td> </tr> </table>			PI: John B. Pritchard Galen J. Neufeld	Research Physiologist IGPA Fellow	LP NIEHS Emporia St. Univ., Kan.
PI: John B. Pritchard Galen J. Neufeld	Research Physiologist IGPA Fellow	LP NIEHS Emporia St. Univ., Kan.			
COOPERATING UNITS (if any)					
LAB/BRANCH Laboratory of Pharmacology					
SECTION Marine Pharmacology and Biomedicine Section					
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709					
TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.1	OTHER: 0.4			
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS					
SUMMARY OF WORK (200 words or less - underline key words) Many <u>aquatic</u> animals are highly sensitive to certain <u>xenobiotic</u> compounds. Such organisms are used as <u>models</u> to identify those <u>physiological processes</u> most sensitive to <u>environmental pollutants</u>. Particular emphasis is placed on <u>membrane functions</u> which underlie these processes. We have utilized as model systems the <u>blue crab</u> (<u>Callinectes sapidus</u>) which is extremely sensitive to <u>organochlorine pesticides</u>, particularly at low environmental salinity where its survival depends on its ability to <u>osmoregulate</u>, and the <u>rock crab</u> (<u>Cancer irroratus</u>) which lacks the ability to <u>osmoregulate</u>. The objective is to establish the mechanism(s) responsible for the sensitivity of crustacea to <u>organochlorine</u> compounds. Parameters examined include 1) the levels of <u>Na,K-ATPase</u> in the gills after adaptation to various environmental salinities; 2) the ouabain binding characteristics of the enzyme; 3) the activity of <u>Na,K-ATPase</u> enzyme after administration of DDT both <u>in vitro</u> and <u>in vivo</u>; 4) the model of inhibition of the enzyme <u>in vitro</u>; and 5) the ability of the intact animal to maintain <u>hemolymph osmolality</u> and <u>ion levels</u> after intravascular administration of DDT.					

PROJECT DESCRIPTION

METHODS EMPLOYED: Na,K-ATPase is measured in freeze-dried, reconstituted homogenates of gill tissue. Ouabain binding is determined by Millipore filtration. Hemolymph osmolality is measured by vapor pressure osmometry and ion content by flame photometry.

MAJOR FINDINGS AND PROPOSED COURSE: 1) The Na,K-ATPase activity in blue crabs acclimated to 100% seawater was highest in gill pairs 6 and 7. Decreasing the salinity in a stepwise manner produced a progressive increase in Na,K-ATPase activity in these gills. This induction was slightly greater in males than in females. Complete induction for crabs transferred directly to 20% seawater required approximately two weeks. The reverse, i.e., the decrease in enzyme activity when 20% seawater-adapted crabs were transferred to 100% seawater, also required approximately two weeks. Na,K-ATPase activity in the gills of the rock crab, which lacks effective osmoregulatory ability, was much lower than in blue crab gills. There was little difference in activity between gill pairs. Furthermore, there was no induction of Na,K-ATPase when the animals were transferred to 50% seawater. Hemolymph osmolality and sodium concentration conformed to that of the aquatic environment.

2) Blue crabs acclimated to 100% seawater were transferred to 20% seawater 24 hr after an intravascular injection of DDT. The subsequent decrease in hemolymph osmolality occurred more rapidly in DDT-dosed animals than in controls, indicating impaired osmoregulatory ability. However, by 28 hr after the transfer to 20% seawater, there were no significant differences between osmolality, sodium concentration and Na,K-ATPase activity. Analysis of the in vitro inhibition by DDT indicated noncompetitive inhibition with ATP as substrate.

The Na,K-ATPase from the gills of rock crabs was highly sensitive to DDT in vitro with significant inhibition at 0.5 ppm. Maximum inhibition of ~ 35% was reached at 9.0 ppm. Additionally, when DDT was injected into the intact animal, there was significant inhibition of Na,K-ATPase when measured as long as 48 hr after injection.

The proposed course is as follows: 1) The biochemical studies of blue crab gill Na,K-ATPase will be continued with primary focus on the ouabain binding characteristics and kinetics in microsomal preparations. Following these determinations, ouabain binding levels will be compared with enzyme activity after salinity changes to determine if the changes in Na,K-ATPase activity with a decrease in salinity represent activation of latent enzyme or synthesis of new enzyme protein.

3) Gills from blue crabs acclimated to different environmental salinities will be processed for electron microscopy to correlate gill ultrastructure with osmoregulatory ability.

4) Studies of DDT inhibition in whole homogenates have shown this to be non-competitive inhibition when ATP was the substrate varied. These studies will be repeated varying the concentration of potassium to determine if the potassium binding site is susceptible.

5) The Na,K-ATPase in whole homogenates is highly sensitive to DDT in vitro. These studies will be repeated with microsomal preparations in which the specific activity of Na,K-ATPase has been enhanced by the removal of extraneous protein and lipid with deoxycholate.

6) Previous experiments have shown that osmoregulation is only temporarily affected when the blue crabs are given a single intravascular dose of DDT. The response to repeated injections and to chronic exposure via the water in which they live will be measured. In this way the membranes of the gill will be continuously exposed to the chemical.

7) Vanadium is a potent inhibitor of partially purified Na,K-ATPase. Preliminary experiments have shown that the Na,K-ATPase in whole homogenates of crab gill is only slightly inhibited. These experiments will be compared with those of microsomal preparations to determine if whole homogenates contain a substance which protects the enzyme from the metal.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The use of sensitive organisms may permit identification of systems particularly prone to disruption by environmental contaminants. Such studies may then a) point to systems which might also be particularly prone to damage in man and b) serve as indicators or warning systems to the accumulation of contaminants in the environment, particularly the marine environment which serves as a sink for persistent pollutants. The sensitivity of the Na,K-ATPase shown here thus may, in conjunction with the studies proposed above, not only indicate the mechanism of toxicity of organochlorine pesticides in an economically important human food, but it also suggests that the processes linked to Na,K-ATPase activity may be among the earliest and most important affected by DDT.

PUBLICATIONS

Neufeld, G. J. and Pritchard, J. B.: An assessment of DDT toxicity on osmoregulation and gill Na,K-ATPase in the blue crab. Symp. Aquatic Toxicol., Amer. Soc. Testing and Materials. In press.

Neufeld, G. J., Cotton, C. U., and Pritchard, J. B.: DDT in the rock crab: Tissue distribution, metabolism and inhibition of gill Na,K-ATPase. Bull. Mt. Desert Island Biol. Lab. In press.

Neufeld, G. J., Pritchard, J. B., and Cotton, C. U.: Inhibition of calcium-dependent ATPase from mollusc mantle tissue. Bull. Mt. Desert Island Biol. Lab. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80032-02 LP
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Excretion of Xenobiotics by Selected Marine and Terrestrial Species		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: John B. Pritchard Nesko Neskovic Alan G. E. Wilson	Research Physiologist Visiting Fellow Visiting Associate	LP NIEHS LET NIEHS LPK NIEHS
COOPERATING UNITS (if any) Laboratory of Environmental Toxicology; Laboratory of Pharmacokinetics; University of Pennsylvania School of Medicine		
LAB/BRANCH Laboratory of Pharmacology		
SECTION Marine Pharmacology and Biomedicine		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 1.9	PROFESSIONAL: 1.3	OTHER: 0.6
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline k- Marine and terrestrial vertebrates are used to examine the role of organic ion transport in the renal and hepatic excretion of environmental contaminants such as DDT and 2,4-dichlorophenoxyacetic acid (2,4-D). Topics under investigation include examination of 1) the importance of renal and hepatic organic anion transport in the rate of elimination of xenobiotics or their metabolites; 2) the interference of foreign compounds with elimination of endogenous wastes or toxins; 3) the role of intracellular binding proteins, such as glutathione S-transferases, in transport and toxicity of organic ions; and 4) the influence of membrane transport-related cellular accumulation in the development of the xenobiotic toxicity in target organs. The role of transport in the elimination of xenobiotics from specific organs, e.g., brain, as well as from the whole organism, is also characterized.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Transport of organic ions is measured in vitro using the isolated renal tubules of winter flounder and slices of rat and rabbit kidney, lung, or liver; the isolated perfused lung is also used in vitro. Intracellular compartmentalization and binding are determined from efflux studies on the isolated tissue preparations after loading in vitro or in vivo. These studies are complemented by clearance measurements in the intact animals.

The binding properties of glutathione S-transferases and microsomal proteins isolated according to published procedures are determined in vitro for comparison with the intact cell data generated above.

The isolated rabbit choroid plexus is used to examine the interaction of xenobiotics with the transport of endogenous and exogenous compounds from the brain.

MAJOR FINDINGS AND PROPOSED COURSE: 1) Studies in winter flounder, rat, and rabbit demonstrate that organic anion transport, an active membrane process, plays a major role in the elimination of DDA, 2,4-D, and similar compounds from the organism. In addition, both DDA and 2,4-D show competition in vitro and in vivo with other organic anions known to be carried by this system. Sites of such interaction include kidney, liver, lung, and choroid plexus (see below).

2) Detailed examination of the renal handling of the herbicide 2,4-D by the winter flounder demonstrated that the flounder more effectively excretes 2,4-D than any other species thus far examined. The basis for this rapid excretion is threefold. First, 2,4-D is an excellent substrate for the organic anion transport systems in vitro (tissue/medium ratios > 30) and in vivo (clearance 2,4-D 500 times the glomerular filtration rate). Second, the flounder has a renal portal circulation that brings a large fraction of its cardiac output through the kidney. Thus, the kidney is able to clear 2,4-D from 1/3 to 1/2 (500 to 750 ml/hr/kg fish) of the cardiac output. Third, unlike the mammals thus far examined, flounder show much less effective binding of 2,4-D to plasma proteins (70% bound in plasma vs. 89-99% in mammals). The importance of plasma binding was confirmed in vitro and in vivo in studies comparing the handling of 2,4-D with that of DDA, which has comparable transport but is much more effectively bound to plasma proteins (97%).

3) The results of the renal studies predicted that DDA and 2,4-D should inhibit the transport of organic acids from the brain and cerebrospinal fluid by the choroid plexus, which shows similar transport properties to kidney. Indeed, it has now been demonstrated that both compounds, at concentrations as low as 10^{-6} M (i.e., < 1 ppm), inhibit the transport of the serotonin metabolite 5-hydroxy-3-indoleacetic acid (HIAA) by the rabbit choroid plexus in vitro. It has also been shown directly that DDA and 2,4-D are both extremely accumulated ($T/M > 30$) by the choroid plexus in vitro. This uptake is blocked by metabolic inhibitors or by other organic anions such as probenecid. Neither 2,4-D nor

DDA altered the uptake of N-methyl-nicotinamide, an organic cation, by the choroid plexus. Thus, all results are consistent with competition between DDA or 2,4-D and HIAA for the organic acid carrier.

Presentation of DDA or 2,4-D to rats in vivo (25 mg/kg) caused only a slight (10%), though significant, elevation in endogenous HIAA levels in the brain. It appears that the extensive plasma binding of both compounds in the mammal limits the free concentration available in vivo, thus protecting choroid plexus HIAA transport. This is under further investigation. However, it is an interesting possibility that the same phenomenon (plasma binding) that limits the excretion of 2,4-D and DDA by mammals in vivo may also protect them from the toxic effects of these compounds.

4) The transport of organic anions by the lung is also under investigation. Although phenol red (PR) is accumulated ($T/M > 4$) by an energy-dependent mechanism in lung slices, neither 2,4-D nor DDA shows comparable transport. Similarly, p-aminohippurate (PAH) is not transported. Thus, the specificity of uptake appears to be quite different from other sites of organic acid transport. Nevertheless, 2,4-D and DDA do inhibit PR uptake. The mechanism of this interaction is under investigation. Additional studies utilizing the isolated perfused lung are being directed toward assuming the physiological role of this transport and uptake from the airways on movement from blood to airways.

5) We have previously shown that DDA may influence the accumulation of the organic cation tetraethylammonium (TEA) in rat cortical slices. Similar findings have been made by others for 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). This interaction appears to occur at the level of intracellular binding. We have now shown that both DDA and 2,4,5-T cause similar inhibition of TEA uptake in other tissues including choroid plexus and lung. We are developing an isolated membrane vesicle preparation, which we will be able to use to investigate the membrane effects separately from intracellular binding. This preparation in conjunction with isolation of intracellular binding proteins, both cytoplasmic and microsomal, will then allow us to characterize the interaction of xenobiotics with the component parts of the transport and excretion process. This will be the main thrust of our projected research over the next fiscal year.

6) Renal sugar transport was also characterized in vitro and in vivo using the flounder in preparation for examining the influence of xenobiotics which alter renal function on sugar transport. Three distinct carrier-mediated sugar transport systems have been characterized at both the apical (i.e., luminal) and basal faces of the tubule. Surprisingly, 2-deoxy-D-galactose was found to demonstrate net secretion in vivo; this was the first demonstration of tubular secretion of a sugar. (A collaborative project with Dr. A. Kleinzeller at the University of Pennsylvania School of Medicine.)

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: An understanding of the mechanisms controlling the ability of marine organisms to eliminate the many foreign compounds reaching the marine environment is vital in predicting the hazards of subsequent consumption of the species by man. Furthermore, the use of model preparations such as the isolated flounder renal tubule permits rapid assessment of the interaction of xenobiotics with renal function, or in the case of organic anions, such as 2,4-D, with other similar transport sites in the body. The confirmation of 2,4-D and DDA inhibition of choroid plexus transport of a normal, but toxic, brain metabolite in the rabbit is an excellent example of the predictive value of such a model system from the marine environment.

PUBLICATIONS

Pritchard, J. B., Hosterman, S., and Kleinzeller, A.: In vivo renal tubular secretion of 2-deoxy-D-galactose by the winter flounder, *Pseudopleuronectes americanus*. Bull. Mt. Desert Island Biol. Lab 16: 80-83, 1976.

Hosterman, S. and Pritchard, J. B.: Organic acid transport: Utility of in vitro kinetic measurements to predict effectiveness of inhibitors in vivo. Bull. Mt. Desert Island Biol. Lab 16: 55-58, 1976.

Jayarom, H. N., Anandaraj, M. P. J. S., Pritchard, J. B., and Guarino, A. M.: Cytotoxicity of plasma and tissue homogenates of sharks on murine lymphoblasts in culture. Bull. Mt. Desert Island Biol. Lab 16: 58-60, 1976.

Pritchard, J. B., Karnaky, K. J., Jr., Guarino, A. M., and Kinter, W. B.: Renal handling of the polar DDT metabolite, DDA (2,2-bis[p-chlorophenyl]acetic acid, by marine fish. Amer. J. Physiol. 233: F126-F132, 1977.

Pritchard, J. B., Booz, G., and Kleinzeller, A.: Renal sugar transport in the winter flounder: V. Secretion of 2-deoxy-D-galactose. Amer. J. Physiol. In press.

Pritchard, J. B.: Kinetic analysis of the renal handling of 2,2-bis(p-chlorophenyl)acetic acid (DDA) by rat kidney in vitro. J. Pharmacol. Exptl. Therap. In press.

Pritchard, J. B. and Cotton, C. U.: Evaluation of the renal handling of 2,4-D by the winter flounder. Bull. Mt. Desert Island Biol. Lab 17: In press.

Booz, G. W., Pritchard, J. B., and Kleinzeller, A.: The renal clearance of D-mannose in the winter flounder. Bull. Mt. Desert Island Biol. Lab 17: In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80036-01 LP			
PERIOD COVERED January 1, 1978 to September 30, 1978					
TITLE OF PROJECT (80 characters or less) Control of Development of Drug Metabolizing Enzymes					
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT					
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%; vertical-align: top;"> PI: Julian E. A. Leakey James R. Fouts </td> <td style="width: 33%; vertical-align: top;"> Visiting Fellow Chief </td> <td style="width: 33%; vertical-align: top;"> LP NIEHS LP NIEHS </td> </tr> </table>			PI: Julian E. A. Leakey James R. Fouts	Visiting Fellow Chief	LP NIEHS LP NIEHS
PI: Julian E. A. Leakey James R. Fouts	Visiting Fellow Chief	LP NIEHS LP NIEHS			
COOPERATING UNITS (if any)					
LAB/BRANCH Laboratory of Pharmacology					
SECTION Toxication-Detoxication					
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709					
TOTAL MANYEARS: 3.2	PROFESSIONAL: 1.5	OTHER: 1.7			
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS					
SUMMARY OF WORK (200 words or less - underline key words) An organism's capacity to metabolize drugs and environmental toxins is limited in the perinatal period due to low levels or absence of the enzyme systems responsible. The purpose of this study is to (1) elucidate the natural factors controlling the development of detoxicating enzyme activity and (2) investigate their mechanisms of action. Enzyme activities of special interest are those dependent upon cytochrome P-450. Work completed has demonstrated that the rate of development of cytochrome P-450 concentrations in neonatal rat liver may be increased by glucocorticoid hormones but not by glucagon or tri-iodothyronine. Glucocorticoids, however, fail to stimulate cytochrome P-450 development in fetal rat liver.					

PROJECT DESCRIPTION

METHODS EMPLOYED: The natural maturation of perinatal rat liver detoxicating capacity is followed by the assay of microsomal and cytoplasmic enzymes responsible on a wt. protein, wt. liver, and wt. DNA basis. Spectrophotometric, fluorometric, and radiometric techniques are used for such assays. The rates of development of such enzymes are manipulated by hormonal treatment (by intraperitoneal injection in the neonate and fetuses exposed by maternal laparotomy) or by removal of endocrine organs (neonatal adrenalectomy). The mechanisms of glucocorticoid hormone action on developing enzymes are investigated by the assay of the specific glucocorticoid cytoplasmic binding protein(s) in perinatal liver and the study of the glucocorticoid-binding, protein complex with isolated nuclei and chromatin.

MAJOR FINDINGS AND PROPOSED COURSE: Work completed so far has determined that 1) the rate of neonatal development of cytochrome P-450 and its dependent enzyme activities may be enhanced by the glucocorticoids, dexamethasone, and cortisol but not by glucagon or tri-iodothyronine.

2) The response of cytochrome P-450 to dexamethasone is slower and requires higher doses than that of the glucocorticoid-responsive enzyme tyrosine aminotransferase.

3) Adult liver cytochrome P-450 concentrations are less responsive to dexamethasone stimulation than those of neonatal liver. This could be in part due to an increased rate of clearance of dexamethasone in the adult.

4) Maternal dexamethasone treatment fails to increase cytochrome P-450 concentrations in fetal liver.

5) Treatments with labeled dexamethasone and binding protein assays have shown that this non-responsiveness of fetal liver is not due to the inability of the dexamethasone to reach the fetal hepatocyte or to lack of fetal glucocorticoid binding protein.

Future work will 1) determine the effect of neonatal adrenalectomy on cytochrome P-450-development; 2) attempt to correlate the degree of dexamethasone-responsiveness of cytochrome P-450 concentrations with the rate of turnover of cytochrome P-450 at various ages; 3) attempt to precociously stimulate cytochrome P-450 development in the fetus by hormone treatment in utero; 4) search for any differences, between fetal and neonatal liver, in glucocorticoid interactions with cytoplasmic binding proteins, nuclei, and chromatin and attempt to correlate such differences with the lack of dexamethasone responsiveness of fetal cytochrome P-450 development.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM AT THE INSTITUTE: Knowledge of the factors controlling development of detoxicating/toxicating enzymes is of great importance to perinatal pharmacology, teratology, and endocrinology. Their immature detoxicating capacity renders perinates

vulnerable to potentially toxic compounds of both endogenous (bilirubin, steroids) and environmental (drugs, pollutants) origin. Delayed development, or overloading of partly developed detoxicating capacity, may result in dangerous pathological conditions (e.g., neonatal jaundice). Alterations in the rate of development of detoxicating enzymes, moreover, can make neonatal drug therapy difficult as large differences in a given drug's potency may arise. Knowledge of the natural factors controlling detoxicating enzyme development would greatly facilitate the manipulation of the rate of development of these enzymes where such pathological conditions necessitate it. The investigation of the mechanism of glucocorticoid action on perinatal liver enzymes associated with this project has wider applications as a potentially valuable tool in the elucidation of the control of eukaryotic gene expression and carcinogenesis.

University of Arizona
Tucson, Arizona
NIH-N01-ES-2111

TITLE: "Extrapolation of PCB Deposition"

CONTRACTOR'S PROJECT DIRECTOR: I. Glenn Sipes, Ph.D.

PROJECT OFFICER (NIEHS): H. B. Matthews, Ph.D., Research Chemist, LPK

DATE CONTRACT INITIATED: April 15, 1977

CURRENT ANNUAL LEVEL: \$194,000

PROJECT DESCRIPTION

OBJECTIVES: The objective of this project is to obtain information on the disposition of polychlorinated biphenyls (PCBs) in dogs and monkeys and to use this knowledge in the further development of a pharmacokinetic model which will predict for a number of species the distribution, accumulation, and excretion of PCBs and other chlorinated xenobiotics after different dose levels and routes of exposure.

METHODS EMPLOYED: The distribution, accumulation, metabolism, and excretion of ¹⁴C-labeled PCBs (4,4'-dichloro-, 2,4,5,2',4',5'-hexachloro-, 2,3,6,2',3',6'-hexachloro-biphenyl) will be studied in Beagle dog and Fasciulus monkey. All major tissue and excreta (urine and feces) are sampled at various times after administration of the PCB. Biliary studies are also to be conducted to determine the necessary pharmacokinetic parameters needed for modeling. Flow rate to the major organs will be determined in the monkey by means of microsphere technique.

MAJOR FINDINGS AND PROPOSED COURSE: 1) The half-life of a PCB in the dog or the monkey is inversely proportional to the rate of metabolism of that PCB.

2) The dog is much better able to metabolize PCBs than is the monkey or even the rat.

3) Chlorination in the para position greatly inhibits PCB metabolism by the monkey, but not by the dog.

4) PCBs with adjacent unsubstituted meta and para positions are readily metabolized by the monkey.

5) The major tissue depots for unmetabolized PCBs are the fat and skin in both the monkey and the dog.

The proposed course is as follows: 1) The amounts of parent PCB vs. metabolites will be determined for each PCB in the tissue and excreta of each species at several time points.

2) An effort will be made to determine the molecular structure of each of the major metabolites of each PCB.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Chlorinated xenobiotics are among the most persistent of our environmental contaminants. These compounds are known to cause a variety of biological disorders in man as well as being at least partially responsible for the declining numbers of certain species of wildlife. Accumulation of high concentrations in the tissues of animals exposed to relatively low doses is a characteristic of certain chlorinated xenobiotics. Yet many of these compounds are used in our current methods of food production, disease control, and certain industrial processes. Thus, we need to be able to predict what will be the disposition of these compounds in animals and man. Understanding the pharmacokinetics of PCBs in different species will help in this prediction or extrapolation of animal data to man.

Virginia Commonwealth University
Richmond, Virginia
N01-ES-6-2117

TITLE: "A Study of the Pharmacokinetics and Toxicity of Kepone in Humans and Laboratory Animals"

CONTRACTOR'S PROJECT DIRECTOR: Philip S. Guzelian, M.D.

PROJECT OFFICER (NIEHS): H. B. Matthews, Ph.D., Research Chemist, LPK

DATE CONTRACT INITIATED: December 15, 1975

CURRENT ANNUAL LEVEL: \$34,997

PROJECT DESCRIPTION

OBJECTIVES: The recent discovery of accidental chronic exposure of a small community to Kepone has presented many problems relating to immediate toxicity and unknown long-term consequences. The goal of this proposal is to conduct a rapid, orderly, and systematic investigation of the absorption, distribution, biotransformation, and excretion of Kepone in humans and animals to aid in understanding its toxicity and to provide a basis for rational therapy.

METHODS EMPLOYED: 1) Human Investigations. Initial clinical findings will be expanded to include tabulation of complete historical, epidemiologic, and physical findings in 25-30 symptomatic patients. Routine clinical lab tests will be done. Blood, saliva, and excreta will be analyzed for Kepone and metabolites. 2) Analytical Methods. Various types of solvent extractions will be used to remove Kepone and metabolites from biological samples, followed by TLC and GLC analysis. 3) Lab Animal Investigations. The relative rates and extent of Kepone excretion in urine, bile, and feces will be compared in different species by the usual methodologies.

MAJOR FINDINGS AND PROPOSED COURSE: Industrial workers exposed to the organo-chlorine pesticide, chlordecone (Kepone), had signs of toxicity in several organs. The extent of toxicity was proportional to the levels of this chemical in the tissues. In 22 patients, chlordecone was eliminated slowly from blood (half-time of 165 ± 27 days -- mean $\pm$ S.E.M.) and fat (half-time of 125 days with a range of 97 to 177), chiefly in the stool. Output of chlordecone in bile was 10 to 20 times greater than in stool, suggesting that chlordecone is reabsorbed in the intestine. Cholestyramine, an anion-exchange resin that binds chlordecone, increased its fecal excretion by seven times. In a five-month trial, cholestyramine significantly accelerated elimination of chlordecone from blood, with a half life of 80 ± 4 days (S.E.M.) ($P < 0.005$) and fat

(half-life of 64 days, with a range of 52 to 85) ($P < 0.05$) when compared to subjects not treated with the resin. Cholestyramine offers a practical means for detoxification of persons exposed to chlordecone and possibly to other lipophilic toxins.

The proposed course is as follows: 1) The patients will be monitored for an indefinite period of time to record the progress of the symptoms of Kepone intoxication and the general health of each exposed individual.

2) The metabolism of Kepone will be studied in fecal samples collected from the exposed humans as well as in monkeys and rodents treated in the laboratory.

3) An effort will be made to investigate the enterohepatic circulation of other lipophilic xenobiotics and to evaluate a series of drugs which might act to interrupt the cycle of enterohepatic circulation and facilitate the excretion of these compounds.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A recent discovery of accidental industrial poisoning of workers with the pesticide Kepone and the possibility of contamination of the air, water, and sewage of a small Virginia community with this chlorinated hydrocarbon has created a need to rapidly develop biologic information regarding this agent. The proposed interdisciplinary studies will investigate the toxic and pharmacokinetic properties of Kepone using a combined approach of in vivo and in vitro systems including man. A well-conducted analysis of this problem may not only yield valuable information on Kepone but may set up a protocol for the investigation of similar incidents of environmental contamination which may occur in the future with other compounds.

PUBLICATIONS

Boylan, J. J., Egle, J. L., Guzelian, P. S.: Use of cholestyramine as a new therapeutic approach for chlordecone (Kepone^R) poisoning: Science 199: 893-896, 1977.

Blanke, R. V., Fariss, M. W., Griffith, F. D.: Analysis of chlordecone (Kepone^R) in biological specimens. J. Anal. Toxicol. 1: 57-62, 1977.

LABORATORY OF PHARMACOKINETICS

LABORATORY OF PHARMACOKINETICS

Summary Statement

The Laboratory of Pharmacokinetics has the responsibility for planning and developing a focus at NIEHS for pharmacokinetic studies and collaborative efforts applying pharmacokinetic principles to enable an understanding of where and why chemicals accumulate in living organisms and how these uptake, storage, and elimination processes are regulated or can be altered. To carry out this mission, Laboratory scientists plan and conduct studies to develop pharmacokinetic models for specific classes of chemicals in various species, and to develop strategies for extrapolating chemical behavior and toxicity from one species to another and to man. They plan and conduct studies on use of pharmacokinetic data to design better treatments for toxicities due to persistent chemicals, to evaluate body burdens of environmental chemicals, and to assess effects of different rates of exposure to environmental chemicals on toxicological outcomes. Scientists evaluate the role of metabolism in chemical accumulations across various species. Finally, they establish and maintain liaison with various groups in and out of government using pharmacokinetic approaches in toxicological evaluations.

There are currently no formal sections in the Laboratory. Rather, there are three small workgroups conducting research in separate but complementary areas all aimed at meeting the objectives of the Laboratory.

Whole Body Disposition Group

This group focuses on measuring whole body disposition of chemical pollutants, and uses the information gained to formulate and validate mathematical models for extrapolation. Through continuing collaboration with the Biometry Branch, and with the Biomedical Engineering and Instrumentation Branch, DRS, NIH, flow-limited pharmacokinetic models were developed for several polychlorinated biphenyl (PCB) isomers in the rat and mouse. The biochemical and physiological parameters used in these models were determined from studies in bile-cannulated animals, isolated perfused liver studies, and whole animal disposition studies as well as from literature values. The model developed was tested and after subsequent refinements, the results indicated that such mathematical models based on physiological concepts could provide an operational framework for interspecies extrapolation of pharmacokinetic profiles.

The model was further defined for the pharmacokinetics of 3,3',5,5'-tetrachlorobiphenyl (4-CB) and the data were used to elucidate the relative importance of degree of chlorination vs. position of chlorination on the biphenyl rings in the overall kinetics of PCB disposition. The results confirmed the previous findings that chlorine position is more important than degree of chlorination because the former can alter the availability of reactive hydrogens thought to facilitate rapid PCB metabolism to excretable metabolites by mammals.

Man and animals usually are exposed chronically to PCBs and other persistent environmental contaminants. Therefore, groups of mice were given chronic oral doses of several PCBs for a period sufficient to produce a steady-state, whereby the rate of excretion equaled the rate of ingestion, or for a period sufficient to provide the data from which the time required to reach a steady-state and corresponding body burden could be calculated. The data obtained were used to simulate the disposition of PCBs encountered in long-term, low-dose exposures.

The liver is the most important site in the body for metabolism and excretion of PCBs as it is for many foreign chemicals. The biliary excretion and enterohepatic circulation of 4-chloro (1-CB) and 2,3,6,2',3',6'-hexachlorobiphenyl (6-CB) and their hydroxylated metabolites were studied in the rat. It was shown that although initially both 1-CB and 6-CB were excreted to a similar extent in the bile, the ultimate excretion of their hydroxylated metabolites after reabsorption from the intestine varied markedly, being entirely biliary for the 6-CB metabolite contrasted with about 50% urinary excretion for the 1-CB metabolite.

In a study done under contract with the University of Arizona, information was obtained on the disposition of several PCBs in dogs and monkeys. It was found that the dog is much better able to metabolize PCBs than is the monkey or even the rat, and that chlorination in the para position greatly inhibits PCB metabolism by the monkey, but not by the dog. As in rodents, half-life of a PCB in the body is inversely proportional to the rate of metabolism of that PCB in either species studied and the major tissue depots are the fat and skin. The data obtained from these higher species are currently being fitted to our models as an important next step toward our objective of trying to extrapolate pharmacokinetic data from lower animals to man.

In another study done under contract with the Medical College of Virginia (MCV) industrial workers intoxicated with the organochlorine pesticide, chlordecone (Kepone[®]), were treated with cholestyramine, an anion-exchange resin that binds Kepone. Previous studies from MCV and our laboratory had shown that Kepone is excreted in the bile of animals and persists in the body due to reabsorption from the gut. Cholestyramine treatment proved to be successful in increasing fecal excretion in humans and, thus, offers a practical means for detoxification of persons exposed to Kepone and possibly other fat-soluble toxins. Preliminary results also indicated an improvement in clinical symptoms in those subjects treated with cholestyramine.

Isolated Organ Workgroup

This group focuses on studies of uptake, storage, metabolism, and release of pollutants and model compounds by isolated perfused organs with present emphasis on the lung. The isolated perfused rodent lung (IPL) system allows examination of the pharmacokinetics of chemicals localizing in the lungs. It also allows examination of the interaction between pollutants and endogenous chemicals in the lungs without interference from other organs and tissues. Past emphasis has been on studying kinetics of chemicals reaching the lungs through the circulation. With the development of techniques for exposing the perfused lung system to inhaled chemicals, emphasis is being placed also on

evaluating the disposition of chemicals reaching the lungs from the inhaled air. As part of an investigation of the effects of inhaled chemicals on the pulmonary clearance of bioactive endogenous compounds, the effect of inhalation exposure of the IPL to the industrial solvent morpholine on the pulmonary clearance of 5-hydroxytryptamine (5-HT) was examined. Morpholine was found to be a potent inhibitor of the enzyme (MAO) which inactivates 5-HT. As might be expected, a significant decrease in the pulmonary clearance of 5-HT was observed. Other environmentally important chemicals which enter the body by the inhalation route will be studied for their effects on the pulmonary pharmacokinetics of endogenous and foreign chemicals.

Classical Pharmacokinetics Workgroup

Classical compartmental pharmacokinetics theories were applied to several environmental agents. A compartmental model was developed to describe the plasma, urinary, and fecal time courses of the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in the rat. The model incorporates both saturable unidirectional renal transport and enterohepatic recycling to describe the pharmacokinetic profile.

Similarly, a novel compartmental model was developed to describe pharmacokinetics of phenolphthalein, a model drug for enterohepatic recirculation. This model may be extrapolated to several compounds of environmental concern for which enterohepatic recycling is an important aspect of their disposition.

The pharmacokinetics of diphenylhydantoin (DPH) and a hexachlorobiphenyl isomer (HCB) were studied to test the theory that a single radioactive dose can be superimposed at the end of chronic toxicity studies to determine possible changes in a chemical's pharmacokinetic characteristics due to either toxic effects or alterations in disposition. The kinetics of DPH, a simple two-compartment chemical, were not altered. However, the kinetics of HCB, a complex multicompartment compound, were altered indicating that classical compartmental models may be less appropriate for substance such as the PCBs, which are substantially redistributed in the body.

Studies were conducted to determine the effects of passive immunization on the distribution of selected PCBs in the mouse. Results indicated that antisera to PCBs were able to increase the amounts of PCBs in plasma and decrease the amounts in tissues of animals previously dosed with PCBs. Further studies are required to determine whether passive immunization is a practical technique for detoxification procedures and/or reduction of toxicant accumulation in animals and man.

Relation of the Pharmacokinetics Program to the Mission of NIEHS

In a broad sense, the NIEHS mission is to detect, prevent, and modify environmentally induced disease through identifying and understanding environmental stressors and their combinations. Before we can prevent or modify the toxic effects of environmental chemical agents, we must be able to determine how and at what rates chemicals enter the body, and how and at what rates the body handles the chemicals in terms of distribution, storage, metabolism, and excretion. From studies on the rates and mechanisms of chemical disposition, we can better understand how and where chemicals exert their toxic effects, and how and why there are qualitative and quantitative differences among

organs and tissues and between species in pharmacologic-toxicologic effects. Hopefully, from such information, we will be able to extrapolate experimental findings from animal test systems across species and ultimately to man.

Personnel

During the year, the Pharmacokinetics Section of the Laboratory of Pharmacology was made a separate organizational unit in the Intramural Program, and re-named as the Laboratory of Pharmacokinetics. Until a permanent laboratory chief can be recruited, Dr. L. G. Hart, Assistant to the Scientific Director, has assumed the responsibility of Acting Chief.

Additions to the Laboratory were: Visiting Scientist -- P. C. Hirom, and biological laboratory technician -- W. C. White (with Drs. Colburn and Hirom).

Lost from the Laboratory was: Staff Fellow -- W. Colburn.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80014-05 LPK												
PERIOD COVERED October 1, 1977 to September 30, 1978														
TITLE OF PROJECT (80 characters or less) Metabolism of Polychlorinated Biphenyls														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0"> <tr> <td>PI:</td> <td>Hazel B. Matthews</td> <td>Research Chemist</td> <td>LPK NIEHS</td> </tr> <tr> <td></td> <td>Shigeru Kato</td> <td>Visiting Fellow</td> <td>LPK NIEHS</td> </tr> <tr> <td></td> <td>James D. McKinney</td> <td>Research Chemist</td> <td>EBCB NIEHS</td> </tr> </table>			PI:	Hazel B. Matthews	Research Chemist	LPK NIEHS		Shigeru Kato	Visiting Fellow	LPK NIEHS		James D. McKinney	Research Chemist	EBCB NIEHS
PI:	Hazel B. Matthews	Research Chemist	LPK NIEHS											
	Shigeru Kato	Visiting Fellow	LPK NIEHS											
	James D. McKinney	Research Chemist	EBCB NIEHS											
COOPERATING UNITS (if any) Environmental Biology and Chemistry Branch														
LAB/BRANCH Laboratory of Pharmacokinetics														
SECTION														
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709														
TOTAL MANYEARS: 1.1	PROFESSIONAL: 1.1	OTHER: 0												
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (X) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline key words) Metabolism to more polar compounds is a prerequisite to polychlorinated biphenyl (PCB) excretion by higher animals. The retention of PCBs is inversely proportional to the rate of metabolism. It is the position and not necessarily the degree of chlorination that determines the rate of PCB metabolism. Two adjacent, unsubstituted carbon atoms are required for a rapid rate of PCB metabolism because their presence facilitates oxidative metabolism, probably via an arene oxide intermediate. However, PCBs without two adjacent unsubstituted carbon atoms may be metabolized via an arene oxide intermediate by shifting a chlorine, using the NIH shift, or by directly inserting a hydroxyl group into the meta position. The major metabolites of PCBs are mono- and di-hydroxylated derivatives which are excreted as glucuronide conjugates.														

PROJECT DESCRIPTION

METHODS EMPLOYED: The studies have utilized ^{14}C -labeled PCBs and *in vivo* metabolism by the rat to obtain the necessary metabolites. The metabolites were isolated by various extraction techniques, purified by thin-layer and column chromatography, and identified by mass spectral and nuclear magnetic resonance analysis. A second approach which is being utilized in the study of the very slowly metabolized PCBs is the synthesis of the anticipated metabolites. These "synthetic metabolites" are then used to identify the authentic metabolites by cochromatography, comparison mass spectrometry, and isotope dilution.

MAJOR FINDINGS AND PROPOSED COURSE: 1) The major metabolites of the PCBs are mono- and di-hydroxylated PCBs, and there is a possibility of an arene oxide intermediate. Metabolism appears to be a prerequisite to PCB excretion. Two adjacent, unsubstituted carbon atoms are necessary for an appreciable rate of metabolism of the PCBs.

2) The major metabolites of 4-chlorobiphenyl have been isolated and identified as: 4-hydroxy-4'-chlorobiphenyl; 3-hydroxy-4'-chlorobiphenyl; 3,4-dihydroxy-4'-chlorobiphenyl; and 3-hydroxy-4-methoxy-4'-chlorobiphenyl. These metabolites account for approximately 60, 7, 23, and 3 percent, respectively, of the radioactivity extracted from the excreta of treated rats.

3) The major metabolite (90%) of 4,4'-dichlorobiphenyl has been isolated and identified as 4-hydroxy-3,4'-dichlorobiphenyl. Traces of the 3-hydroxy-4,4'-dichlorobiphenyl have also been isolated.

4) The metabolites of 2,4,5,2',5'-pentachlorobiphenyl have been described and are 3-hydroxy-2,4,5',4',5'-pentachlorobiphenyl, 3,4-dihydro-3,4-dihydroxy- and 3,4-dihydroxy-pentachlorobiphenyl. Only approximately 13% of this compound is excreted as the parent compound; 60% of the radioactivity excreted was in the form of the monohydroxy; and about 25% was in the form of the dihydrodiol and dihydroxy metabolites.

5) The metabolites of four hexachlorobiphenyls have been isolated and identified. The parent PCBs are 2,4,5,2',4',5'-hexa-, 2,4,6,2',4',6'-hexa-, 2,3,6,2',3',6'-hexa- and 2,3,5,2',3',5'-hexa-chlorobiphenyl. Each of these PCBs, with the exception of 2,3,6,2',3',6'-hexachlorobiphenyl, is very slowly metabolized and excreted. 2,3,6,2',3',6'-Hexachlorobiphenyl is very rapidly metabolized and excreted. The different rates of metabolism are attributed to the presence or absence of two adjacent, unsubstituted carbon atoms. Only 2,3,6,2',3',6'-hexachlorobiphenyl has two adjacent, unsubstituted carbon atoms.

6) In the absence of adjacent, unsubstituted carbon atoms, the greater number of equivalent open meta positions, the greater the metabolism, probably by direct insertion of a hydroxyl group.

The proposed course is as follows: 1) The toxicology and other biological effects of the synthetic metabolites will be the subject of further investigation.

2) An attempt will be made to synthesize one or more arene oxide intermediates of these PCB metabolites, and, if possible, to study the toxicology of these metabolic intermediates.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The polychlorinated biphenyls are ubiquitous environmental contaminants, and knowledge of their biotransformation and the identification of their metabolites is essential to our understanding of their importance as potential hazards to man and his environment. We have evidence that arene oxides are formed in the metabolism of PCBs. Arene oxides have been implicated in a number of undesirable biological reactions including teratogenesis, carcinogenesis, and tissue necrosis.

PUBLICATIONS

Hass, J. R., Jao, L. T., Wilson, N. K., and Matthews, H. B.: Metabolism of 4-chlorobiphenyl and 4,4'-dichlorobiphenyl in the rat: Qualitative and quantitative aspects. J. Agr. Food Chem. 25: 1330, 1333, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80015-05 LPK												
PERIOD COVERED October 1, 1977 to September 30, 1978														
TITLE OF PROJECT (80 characters or less) Transport of Xenobiotics by Blood and Its Constituents														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI:</td> <td style="width: 33%;">Hazel B. Matthews</td> <td style="width: 33%;">Research Chemist</td> </tr> <tr> <td></td> <td>Marshall W. Anderson</td> <td>Mathematician</td> </tr> <tr> <td></td> <td></td> <td>LPK NIEHS</td> </tr> <tr> <td></td> <td></td> <td>LPFT NIEHS</td> </tr> </table>			PI:	Hazel B. Matthews	Research Chemist		Marshall W. Anderson	Mathematician			LPK NIEHS			LPFT NIEHS
PI:	Hazel B. Matthews	Research Chemist												
	Marshall W. Anderson	Mathematician												
		LPK NIEHS												
		LPFT NIEHS												
COOPERATING UNITS (if any)														
LAB/BRANCH Laboratory of Pharmacokinetics; Laboratory of Pulmonary Function and Toxicology														
SECTION														
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709														
TOTAL MANYEARS: 0.1	PROFESSIONAL: 0.05	OTHER: 0.05												
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td>☐ (a) HUMAN SUBJECTS</td> <td>☐ (b) HUMAN TISSUES</td> <td>☒ (c) NEITHER</td> </tr> <tr> <td colspan="3">☐ (a1) MINORS ☐ (a2) INTERVIEWS</td> </tr> </table>			☐ (a) HUMAN SUBJECTS	☐ (b) HUMAN TISSUES	☒ (c) NEITHER	☐ (a1) MINORS ☐ (a2) INTERVIEWS								
☐ (a) HUMAN SUBJECTS	☐ (b) HUMAN TISSUES	☒ (c) NEITHER												
☐ (a1) MINORS ☐ (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline key words) The polychlorinated biphenyls (PCBs) and certain chlorinated pesticides are readily taken up by the <u>blood</u> and its components and transported to the <u>liver</u> where they are readily released. Our studies suggest that these compounds are distributed between the <u>red blood cells</u>, <u>albumin</u>, and <u>lipoproteins</u> at ratios which vary according to the <u>lipophilicity</u> of the chlorinated hydrocarbon involved. Thus, the process is thought to be a passive one in which the physical and chemical properties of the chlorinated hydrocarbons determine the blood constituent in which they will be most concentrated.														

PROJECT DESCRIPTION

METHODS EMPLOYED: The distribution of ^{14}C -labeled xenobiotics such as biphenyl, eight different polychlorinated biphenyls (PCBs), three chlorinated pesticides, one nonchlorinated pesticide, and an endogenous compound, a steroid hormone, are being studied in various blood fractions. Methods employed included adsorption and molecular sieve chromatography, density gradient centrifugation, and electrophoresis.

MAJOR FINDINGS AND PROPOSED COURSE: We have investigated the nature of the association between PCBs and blood components. Both *in vivo* or *in vitro* PCBs are associated with red blood cells (RBCs), albumin, and lipoproteins. The fraction associated with RBCs is most rapidly removed from the blood by the tissues while the fractions associated with the plasma proteins are released more slowly. Gel filtration of plasma containing PCB showed that the lipoprotein fraction contained a higher specific activity (dpm/mg of protein) than did albumin. Other methods of plasma fractionation, e.g., chromatography on glass powder columns and gradient centrifugation, have confirmed the affinity of the PCBs for the lipoproteins and have shown that the relative amounts of a given PCB associated with the lipoproteins and the albumin is related to the lipophilicity of the given PCB. The results of these studies imply that the association between PCBs and blood proteins is nonspecific and that the factors controlling the relative amounts of PCB associated with the different classes of proteins are biophysical rather than biochemical.

We have shown no evidence that the transport of lipophilic xenobiotics interferes with the normal transport of endogenous materials.

We think that the project has been investigated in sufficient detail to confirm that the transport of lipophilic xenobiotics by blood is by passive and nonspecific association of these compounds with blood components. Furthermore, under normal circumstances the transport of such xenobiotics by blood will not interfere with the transport of endogenous compounds. Thus, the results of this project are currently being prepared for publication. Upon publication of the results, the project will be terminated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Environmental exposure of man to a large number of xenobiotics via air, water, or food contamination is significant. These materials are transported by the blood to various detoxication, excretion, or storage sites within the body. Thus, if the transport of any one of these materials should interfere with the normal transport of endogenous materials by the blood then it could result in an imbalance of homeostasis and serve as a mechanism of toxic effect.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80016-05 LPK
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Pharmacokinetics of Chlorinated Xenobiotics		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Hazel B. Matthews Other: Nydia M. Morales Daniel B. Tuey Robert L. Dedrick Robert Lutz Peter M. Bungay	Research Chemist Visiting Fellow Staff Fellow Section Chief Chemical Engineer Chemical Engineer	LPK NIEHS LPK NIEHS BB NIEHS BEIB DRS BEIB DRS BEIB DRS
COOPERATING UNITS (if any) Biometry Branch, NIEHS; Chemical Engineering Section, BEIB, DRS, NIH		
LAB/BRANCH Laboratory of Pharmacokinetics		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 3.9	PROFESSIONAL: 2.5	OTHER: 1.4
CHECK APPROPRIATE BOX(ES)		
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER		
☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline key words) Polychlorinated biphenyls (PCBs), 2,4,5,2',4',5'-hexabromobiphenyl (PBB), and Kepone are readily absorbed from the gut, rapidly removed from the blood, and initially stored in the liver and muscle. At later time points the less chlorinated PCBs are metabolized and excreted while the highly chlorinated PCBs and the PBB are translocated from the liver and muscle to the skin and adipose tissue which are the sites of long-term storage. The degree of PCB metabolism decreases as the chlorine content increases, and the effect of increasing chlorine content is most marked when the chlorine atoms are arranged so that the biphenyl molecule does not have two adjacent, unsubstituted carbon atoms. The PBB and Kepone do not have two adjacent, unsubstituted carbon atoms and are not subject to an appreciable amount of metabolism. Kepone is stored primarily in the liver and is excreted in the bile at a rate proportional to the liver concentration. On the other hand, the PBB is stored primarily in the adipose tissue, is subject to very little excretion, and thus has an infinite half-life in the male rat. After chronic dosing, the disposition of PCBs after long-term, low-dose exposure was simulated.		

PROJECT DESCRIPTION

METHODS EMPLOYED: The distribution, accumulation, and excretion of eight ^{14}C -labeled PCBs (4-chloro-, 4,4'-dichloro-, 3,5,3',5'-tetrachloro-, 3,4,3',4'-tetrachloro-, 2,4,5,2',5'-pentachloro-, 2,3,6,2',3',6'-hexachloro-, 2,3,5,2',3',5'-hexachloro-, 2,4,6,2',4',6'-hexachloro-, 2,4,5,2',4',5'-hexachloro-biphenyl), Kepone, and a polybrominated biphenyl (PBB) (2,4,5,2',4',5'-hexabromobiphenyl) were studied after iv and oral administration to rats. Four of the PCBs (4-chloro-, 4,4'-dichloro-, 2,4,5,2',5'-pentachloro-, and 2,4,5,2',4',5'-hexachlorobiphenyl) were also studied in the mouse. The major tissues and excreta were sampled at times varying from a few minutes to several weeks post-treatment. Distribution and excretion were studied under conditions of normal feeding and starvation. Analyses were facilitated by the use of a biological materials' oxidizer and liquid scintillation counting. All of the data were subjected to further analysis by computer.

MAJOR FINDINGS AND PROPOSED COURSE: 1) Each of the PCBs was rapidly removed from blood with initial storage primarily in the liver and muscle. Long-term storage was in the skin and adipose tissue with most of the stored material being unchanged compounds. Storage of metabolites in skin and adipose tissue accounted for a negligible portion of the total PCB dose. Where two adjacent carbon atoms were present, the excretion of the PCBs was, in most cases, inversely proportional to their degree of chlorination. One hexachlorobiphenyl (2,3,6,2',3',6'-) was metabolized and excreted quite rapidly. The pattern of distribution and excretion of PCBs studied in the mouse is similar to that observed in the rat except that the rates tend to be more rapid in the smaller animal.

2) Kepone is readily absorbed from the gut, stored primarily in the liver, and is slowly excreted in bile. Kepone excretion in bile is proportional to the Kepone concentration of the liver. The half-life of Kepone in the rat is approximately 18 days. The long half-life is due to the inability of the rat to metabolize Kepone at an appreciable rate and to intestinal reabsorption of much of the unmetabolized Kepone which is excreted in bile. The excretion of Kepone in bile was linear with dose between 0.1 and 10 mg/kg.

3) The gut absorption, distribution, and excretion of the major component of Firemaster BP-6^R, 2,4,5,2',4',5'-hexabromobiphenyl, has been studied in the male rat. This polybrominated biphenyl was readily absorbed from the gut, initially distributed throughout the body, eventually stored primarily in the adipose tissue, was not subject to appreciable metabolism, and was excreted almost exclusively in the feces and at a very slow rate. Approximately 90% of an oral dose was absorbed from the gut and extrapolation of the rate of excretion to infinity indicates that less than 10% of the total dose would ever be excreted.

4) A mathematical model has been developed to describe the kinetics of distribution, metabolism, and excretion of 4-chloro-, 4,4'-dichloro-, 2,4,5,2',5'-pentachloro-, and 2,4,5,2',4',5'-hexachloro-biphenyl in rats given an intra-

venous dose of 0.6 mg/kg. A modified flow-limited model simulates the penta- and hexachloro-biphenyl data for times up to 96 hr but underpredicts the mono- and dichloro-biphenyl data beyond 48 hr. The rate of metabolism by the liver decreases as chlorination increases such that the rate constant is 200 times smaller for the hexachlorobiphenyl than for the monochlorobiphenyl. The value of the biliary clearance of metabolite is nearly the same for each isomer, whereas the value of the urinary clearance decreases with increasing chlorination, being 10 times smaller for hexachlorobiphenyl than for monochlorobiphenyl. The distribution coefficients between most tissues and blood are larger for the parent compound of each isomer than for its metabolites. Hexachlorobiphenyl has the largest distribution coefficient of all isomers in each tissue, whereas the mono-, di-, and penta-chlorobiphenyl show no consistent variation. For each isomer the distribution coefficient is greater in the fat than in any other tissue. Changes in the fat volume of the growing rats were incorporated in the model in order to simulate the hexachlorobiphenyl concentration in blood and fat for 42 days.

5) The mathematical model developed for the rat has been modified for extrapolation to the mouse. The model was adjusted for tissue volumes, perfusion rates, gut transport time, etc., and used to simulate the pharmacokinetics of four PCBs and their metabolites in the mouse. Predicted PCB disposition was compared with experimental data obtained at selected time points for 4-chloro-, 4,4'-dichloro-, 2,4,5,2',5'-pentachloro-, and 2,4,5,2',4',5'-hexachloro-biphenyl (1-, 2-, 5-, and 6-CB, respectively). Tissue and excreta concentration-time profiles obtained from the experimental data generally followed the distribution pattern predicted by the mathematical model. The disposition of these PCBs in mice was similar to that observed in rats. Peak contents in fat were about 10, 15, 40, and 75% of the dose for 1-, 2-, 5-, and 6-CB, respectively. Total excretion was inversely correlated with the degree of chlorination. Excretion in urine was significant for 1- and 2-CB (81 and 57%) but not for 5- or 6-CB. The results suggest that mathematical models based on physiological concepts can provide an operational framework for interspecies extrapolation of pharmacokinetic profiles.

6) The pharmacokinetics of 3,3',5,5'-tetrachlorobiphenyl (4-CB) was studied to determine the importance of chlorine position to the kinetics of polychlorinated biphenyl distribution, metabolism, and excretion by the rat. Tissue samples and excreta were collected at times ranging from 2 hr to 42 days after intravenous injection of a single 0.6 mg/kg dose of ^{14}C -labeled 4-CB. The relative fractions of 4-CB and its metabolites were determined in selected samples, and 4-hr bile cannulations were performed to establish the rate of biliary excretion. A mathematical model description of PCB kinetics in the rat was defined here for 4-CB and used to generate tissue and excretion concentration-time profiles for both the parent compound and its metabolites. Model predictions were in good agreement with the experimental data, and showed how the individual pharmacokinetics of 4-CB compared to several other PCBs. The rate of 4-CB metabolism and excretion was slower than would be predicted according to its degree of chlorination. The relative rates of metabolism for 4-CB and five other differently chlorinated biphenyls could be

ordered according to the number of adjacent unsubstituted carbon atom pairs they contained. It appears that chlorine position is more important than degree because the former can alter the availability of vicinal hydrogens thought to facilitate rapid PCB metabolism by mammals.

7) Groups of mice were administered a chronic oral dose of an individual PCB, 4-chloro-, 4,4'-dichloro-, 2,4,5,2',5'-pentachloro- or 2,4,5,2',4',5'-hexachloro-biphenyl for a period sufficient to produce a steady-state, where the rate of excretion equaled the rate of administration, or for a period sufficient to provide the data from which the time required to reach a steady-state and corresponding body burden could be calculated. One group of animals was sacrificed one day after the last dose and a second group of animals was held for a period sufficient to provide the data necessary for a calculation of the rate of decline from steady-state. The data obtained in this study has been used to simulate the disposition of PCBs encountered in long-term, low-dose exposures.

8) The covalent binding of PCBs to nucleic acids and protein has been quantitated at various time points during chronic feeding studies. The amount of covalent binding has been correlated with the amount of activated metabolites formed from individual PCBs during their metabolism.

The proposed course is as follows: 1) It has been observed that the rate of biliary excretion of a PCB following an iv dose is an excellent indicator of the rate of metabolism. The rate of PCB metabolism has previously been shown to be the most important factor determining the half-life of a PCB. An effort will be made to devise a method which will facilitate a prediction of the long-term disposition of PCBs and other lipophilic xenobiotics, based on data obtained from studies of biliary excretion conducted for only a few hours.

2) The data obtained in the study of the disposition of Kepone will be used to construct a pharmacokinetic model for the disposition of this compound in the rat.

3) A comparison of the biliary excretion of PCBs with the excretion of these compounds in the urine and feces indicates that enterohepatic circulation plays an important role in determining the route of excretion for these compounds. Furthermore, enterohepatic circulation is profoundly affected by the molecular weight of the compound involved. Therefore an effort will be made to delineate the physiological mechanisms that account for these observations.

4) One of the purposes of studying the PCBs is that they serve as such excellent model compounds for the disposition of lipophilic halogenated hydrocarbons. Therefore the disposition of several other types of lipophilic halogenated hydrocarbons will be studied in order to test the pharmacokinetic models which have been devised to predict the disposition of PCBs.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Halogenated xenobiotics are among the most persistent of our environmental contaminants. These compounds are known to cause a variety of biological disorders in man as well as being at least partially responsible for the declining numbers of certain species of wildlife. Accumulation of high concentrations in the tissues of animals exposed to relatively low doses is a characteristic of certain chlorinated xenobiotics. Yet many of these compounds are used in our current methods of food production, disease control, and certain industrial processes. Thus, we need to be able to predict what will be the disposition of these compounds in animals and man. Understanding the pharmacokinetics of halogenated hydrocarbons in different species will help in this prediction or extrapolation of animal data to man and may lead to more specific modes of treatment including better ways to accelerate removal of such compounds from the body.

PUBLICATIONS

Lutz, R. J., Dedrick, R. L., Matthews, H. B., Eling, T. E., and Anderson, M. W.: A preliminary pharmacokinetic model for the disposition of PCBs in rats. Drug Metab. Disp. 5: 386-396, 1977.

Anderson, M. W., Eling, T. E., Lutz, R. J., Dedrick, R. L., and Matthews, H. B.: The construction of a pharmacokinetic model for the disposition of polychlorinated biphenyls in the rat. Clin. Pharmacol. Therap. 22: 765-773, 1977.

Tuey, D. B. and Matthews, H. B.: The effect of chlorine position on 3,3',5',5'-tetrachlorobiphenyl in the rat. Drug Metab. Disp. 5: 444-450, 1977.

Matthews, H. B., Kato, S., Morales, N. M., and Tuey, D. B.: The distribution and excretion of 2,4,5,2',4',5'-hexabromobiphenyl, the major component of Firemaster BP-6. Toxicol. Environ. Hlth. 3: 599-605, 1977.

Matthews, H. B., Tuey, D. B., and Anderson, M. W.: Pharmacokinetic models for lipophilic compounds. Environ. Health Persp. 20: 257-262, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 35001-01 LPK
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Disposition and Enterohepatic Circulation of Polychlorinated Biphenyls and Their Metabolites		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Paul C. Hirom Visiting Scientist LPK NIEHS		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Pharmacokinetics		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.5	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline key)		
The biliary excretion and enterohepatic circulation of two polychlorinated hydrocarbons, 4-chlorobiphenyl and 2,3,6,2',3',6'-hexachlorobiphenyl, have been studied in the rat. Both PCBs show similar biliary excretion and absorption from the intestine yet the ultimate route of elimination differs between the two compounds being more urinary than biliary in the case of 4-chlorobiphenyl but entirely biliary for the hexachlorobiphenyl. The rate of elimination of the hydroxylated metabolites of 4-chloro- and 2,2',4,5,5'-pentachlorobiphenyl is more rapid than for the parent compounds especially in the latter case. Again, the ultimate fate of the two compounds differs despite an initially similar extent of biliary elimination. 3'-Hydroxy-2,2',4,5,5'-pentachlorobiphenyl is excreted entirely into the feces, whereas 4'-hydroxy-4'-chlorobiphenyl is eliminated equally in the urine and the feces.		

PROJECT DESCRIPTION

METHODS EMPLOYED: The biliary elimination of a number of ^{14}C PCBs was studied in the rat. Two of these, 4-chlorobiphenyl and 2,3,6,2',3',6'-hexachlorobiphenyl were subjected to a detailed study of enterohepatic circulation. Compounds were administered by intravenous injection to rats with bile duct cannula and bile and urine were collected for suitable time periods and the samples analyzed for ^{14}C content and to identify the major metabolites present. Bile obtained in this manner was infused into the duodenum of other bile duct-cannulated rats and the extent of excretion in the bile and urine determined. At the completion of the experiment the animals were sacrificed and tissues sampled for analysis of residual ^{14}C by sample oxidation and liquid scintillation counting.

The distribution and elimination of 4-hydroxy-4'-chlorobiphenyl and 3'-hydroxy-2,2',4,5,5'-pentachlorobiphenyl were also investigated. These two compounds are the major metabolites of 4-chloro- and 2,2',4,4,4'-pentachlorobiphenyl, respectively.

MAJOR FINDINGS AND PROPOSED COURSE: 1) The rate of elimination of PCBs from the body appears to be governed by their rate of metabolism. The principal route of elimination varies according to the degree of chlorination, the more highly chlorinated PCBs generally showing a greater affinity for fecal elimination. The extent of fecal elimination, however, does not always reflect the extent of biliary elimination. Thus, 4-chlorobiphenyl and 2,3,6,2',3',6'-hexachlorobiphenyl while exhibiting similar extents of biliary elimination (circa 50% of dose in 4 hr) show marked differences in the ultimate route of elimination with 60% of the dose of 4-chlorobiphenyl appearing in the urine whereas only 10% of the dose of 2,3,6,2',3',6'-hexachlorobiphenyl is excreted in the urine.

2) Following the infusion of biliary metabolites into the duodenum, approximately 50% of the dose is absorbed from the intestine within 12 hr. Of the material re-excreted after absorption in the case of 2,3,6,2',3',6'-hexachlorobiphenyl, it all appears in the bile whereas with 4-chlorobiphenyl 33% of that re-excreted appears in the urine and the remainder in the bile; very little of the reabsorbed material is found in the body tissues at the end of the experimental period.

3) The rate of elimination of the hydroxylated biphenyl metabolites is more rapid than with the parent compound. This is particularly marked in the case of 3'-hydroxy-2,2',4,5,5'-pentachlorobiphenyl whose parent compound is quite slowly metabolized in the rat compared to 4-chlorobiphenyl. The major routes of elimination are markedly different for the two compounds, the elimination being split equally between the feces and urine in the case of 4-hydroxybiphenyl and almost entirely fecal in the case of 3'-hydroxy-2,2',4,5,5'-pentachlorobiphenyl. The biliary elimination of these two compounds is, however, not markedly different and blood and tissue levels of the two compounds are similar over a 4-hr time period.

4) It is proposed to investigate the pharmacokinetics of these compounds in more detail particularly with respect to their absorption from the intestine and extraction of the absorbed material from the portal blood by the liver. It is also proposed to develop pharmacokinetic models using the data obtained as has been done in the case of the model compound phenolphthalein, using data obtained before I came to NIEHS.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE : The polychlorinated biphenyls are ubiquitous environmental contaminants which in some cases are persistent in the mammalian organism. Excretion of these compounds in the bile is a significant route of elimination, and it is important to assess the influence of this in determining the pharmacokinetic profiles of these compounds. Marked species differences in biliary elimination are known to occur and a knowledge of this is thus important in the extrapolation of animal data to man.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 35002-01 LPK
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Classical Pharmacokinetics Theory Application		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Wayne A. Colburn Staff Fellow LPK NIEHS		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Pharmacokinetics		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 1.33	PROFESSIONAL: 0.67	OTHER: 0.66
CHECK APPROPRIATE BOX(ES)		
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER		
☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Classical compartmental pharmacokinetics were applied to several environmental agents. A compartmental model was developed to describe the plasma, urinary, and fecal time-course of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in the rat. The model incorporates both saturable unidirectional renal transport and enterohepatic recycling to describe the pharmacokinetic profile. Experiments are underway using ¹⁴ C-2,4,5-T in the rat to elucidate the model. Similarly, a novel compartmental model was developed to describe pharmacokinetics of phenolphthalein, a model drug for enterohepatic recirculation. Experimental compounds and modeling work are being conducted to determine the pharmacokinetics of saccharin in the rat. Dose-dependence and renal mechanisms are being studied. Diphenylhydantoin (DPH) and hexachlorobiphenyl (HCB) were studied to test the theory that a single radioactive dose can be superimposed at the end of chronic toxicity studies to determine possible changes in their pharmacokinetic characteristics due to toxic effects. No effect was seen with DPH whereas HCB kinetics were dissimilar between doses. Passive immunization of mice with antisera to chlorobiphenyls was used to redistribute these compounds back into the plasma. This technique can, in theory, be used to decrease the accumulation of PCBs		

PROJECT DESCRIPTION

METHODS EMPLOYED: Computer and animal experiments were conducted to develop pharmacokinetic theory and to apply it to classical model development and laboratory animal experiments with several environmental and/or model compounds.

MAJOR FINDINGS AND PROPOSED COURSE: 1) Saturable renal excretion and enterohepatic recycling are present in the pharmacokinetics of 2,4,5-T. Further studies are being conducted to determine if it is possible for renal and plasma clearance to be different in the rat.

2) Phenolphthalein (model compound) pharmacokinetics are described using an enterohepatic recirculation model. The pharmacokinetic model developed to describe phenolphthalein plasma concentrations can be extrapolated to several compounds of environmental concern.

3) Saccharin disposition has been studied extensively due to its implication as a bladder carcinogen in the rat. The pharmacokinetics of saccharin and a model for the description of plasma and urine time course in the rat are being developed at present. It appears that dose-dependent pharmacokinetics and unique renal mechanisms are operative in the rat. These findings are being pursued.

4) DPH and HCB have been studied to test the theory that a single radioactive dose at the end of chronic treatment or control periods can establish whether the pharmacokinetic characteristics of a compound have been altered by its own toxicity. DPH, a single two-compartment drug, verified the theory. However, the kinetics of HCB, a complex multicompartment compound, were altered during the test indicating that the theory does not hold for substances that are substantially redistributed in the organism. Further studies are required to bridge the expanse of substances between the two extremes chosen for this study.

5) Studies were conducted to determine the effect of passive immunization on the distribution of monochlorobiphenyl (MCB) and tetrachlorobiphenyl (TCB) in the mouse. Studies indicate that the antisera were able to increase the amount of MCB/TCB in the plasma and decrease the amount in the tissues. Further studies are required to determine whether this technique is generally applicable to other species and other environmental compounds.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: 1) Theory derived from model development can be applied to detoxification procedures in man. Similarly, models developed for specific substances can often be extrapolated to several categories of environmental contaminants.

2) Physiological mechanisms derived from pharmacokinetic theory and application can be used for substantiating or negating extrapolation from animal to man.

3) The use of antisera to environmental contaminants for detoxification procedures or reduction of accumulation is proposed. Immuno-hemoperfusion is an expanded technique arising from this research.

PUBLICATION

Colburn, Wayne A.: A model for dose-dependent pharmacokinetics of chlorophenoxy acid herbicides in the rat: The effect of enterohepatic recycling. J. Pharmacokinet. Biopharm. In press.

LABORATORY OF PULMONARY FUNCTION AND TOXICOLOGY

THE LABORATORY OF PULMONARY FUNCTION AND TOXICOLOGY

Summary Statement

The Laboratory of Pulmonary Function and Toxicology (LPFT) was established during FY 1978. The purpose of this laboratory is 1) to conduct studies on the structure and function of the normal respiratory tract, 2) to investigate mechanisms of injury and repair in respiratory tract tissues exposed to environmental toxins, 3) to study the pathogenesis of specific disorders of pulmonary tissues (e.g. pulmonary fibrosis). The ultimate goal is to obtain new insights into the pulmonary biology and pathology of the lung which will provide the scientific basis for designing rational measures for prevention of pulmonary diseases. To carry out its mission, the Laboratory of Pulmonary Function and Toxicology must encompass a diversity of scientific talents and interests including biochemistry cell biology, immunology, toxicology, and pathology. Presently the LPFT consists of the following groups: endocrinology, prostaglandins, biochemical pathology, cell biology, and environmental carcinogenesis. Personnel for the establishment of a pulmonary pathology group are being hired. The first three are established biochemical groups, with a firm commitment to pulmonary research, which joined the LPFT upon its inception. Cell biology and carcinogenesis are new groups, still in the formative stage.

BACKGROUND AND SCOPE

The respiratory tract is the organ system, bearing the heaviest burden of environmental contamination brought about by urbanization and industrialization. It is the target for numerous gaseous and particulate air pollutants originating from automobiles and industries. In addition, intense occupational exposure of the lungs occur in a wide variety of work environments. We have also learned in recent years that substances entering the body by routes other than the airways (e.g. ingestion of food additives, parenteral administration of pharmaceuticals), may cause severe toxic changes of respiratory tract tissues. As a result of the great variety of exposures (not all of them man-made) acute and chronic respiratory disorders are among the most common diseases with environmental factors as major etiological components. Yet our understanding of the pathogenesis of even the major groups of chronic respiratory diseases: bronchitis, emphysema, pulmonary fibrosis, and bronchogenic carcinoma is still rudimentary. At present, we are neither able to detect these diseases at an early stage of development, when the damage is presumably still reversible, nor are we able in the majority of cases to halt their progression effectively, once the diseases are diagnosed. The reasons for this dilemma are many. One is the often uncertain and apparently complex etiology with multiple interacting factors (individual susceptibility, predisposing injuries or diseases, combination of insults); another is the inadequacy of our basic knowledge of pulmonary biology. The respiratory tract is a complex organ system composed of multiple segments with a great number of cells with diverse functions, many of which are only vaguely understood.

This complexity has made it difficult to investigate the respiratory and non-respiratory functions of the various lung tissues and cells, their unique metabolic characteristics, their life cycle and turnover, their susceptibility to injury, and their capacity for repair. It is clearly evident that new experimental approaches have to be developed to advance our knowledge in the field of pulmonary biology and pathobiology.

Studies in the pulmonary branch will be conducted at various levels of biological organization. The major emphasis, however, will be concentrated at the cellular and biochemical level. The research efforts will encompass studies of normal cell and tissue functions as well as their perturbations by various environmental toxic agents.

PULMONARY ENDOCRINOLOGY

The major research goal of this group is to investigate the hormonal regulation of various lung tissue functions, to identify the sites of hormone secretion, the nature of the hormones involved, the function(s) regulated and the mechanism of regulation. This area of pulmonary research is a new field which is expected to produce many significant discoveries in coming years. Histochemical and ultrastructural studies have shown that the respiratory tract of mammals contains cells which are similar to the neuro-endocrine or entero-chromaffin cells known to occur, e.g., in the digestive tract. The role of these so-called APUD cells (amine precursor uptake and decarboxylation) in the lungs or their secreted products is unknown. It has been suggested that they may have chemoreceptor functions (e.g. O_2 , CO_2 tension) or that they may be related to the regulation of mucus secretion.

A closely related project deals with the hormonal activity of respiratory tract neoplasms. It is well documented that many human bronchogenic carcinomas produce a variety of peptide hormones (serotonin, calcitonin, vasopressin, pro-ACTH, etc.). It is not known, however, whether this is a reflection of the biochemical activity of the progenitor cell(s) from which these tumors derive or rather of the derepression and gene activation believed to be a fundamental feature of carcinogenic events. The occurrence of presumptive hormone secreting cells in the conducting airways and the hormonal activity in tumors arising from these airways (squamous cell carcinoma, oat cell carcinoma, carcinoid) present, an intriguing enigma to the researcher of pulmonary biology and the student of pulmonary carcinogenesis alike.

The studies during the past year have been concerned with 1) identification of hormonal activity in argyrophilic cells of the airways, using a large number of antisera to a variety of polypeptide hormones, 2) demonstration of pro-ACTH and ACTH in chemically induced respiratory tract tumors of rats, 3) attempts to increase the number of APUD cells in hamsters by injection of a carcinogenic nitrosamine and 4) isolation and purification of the prohormone of ACTH from bovine pituitary glands.

Recently, a new project was begun to study the binding and release of bioactive peptides and amines by mast cells. In the lungs, mast cells are commonly found in the submucosal tissues of the airways, i.e., in close proximity to bronchial

muscle as well as to the secretory epithelium. Their role in regulating bronchial smooth muscle tone and mucus secretion by release of substances such as histamine and serotonin has been studied in various laboratories. However, many questions remain unanswered. In the current studies it was found that neurotensin and substance P reversibly bind to rat mast cells and that substance P causes a marked release of histamine from mastocytoma. The release mechanism of bioactive amines from mastocytoma cells is being further investigated. Several polypeptide hormones were detected in extracts from mastocytoma cells, which have not previously been found in mast cells.

PROSTAGLANDIN RESEARCH

This group of investigators is primarily concerned with the metabolism and transport of Prostaglandins (PG) in the lung. Prostaglandins are fatty acids with diverse functions. Among them are broncho constrictors ($\text{PGF}_{2\alpha}$) and bronchodilators (PGE_2). Some of them are suspected to be involved in inflammatory and anaphylactic reactions, and exposure of animals to oxidant gases has resulted in inhibition of prostaglandin metabolism. These fatty acids and their metabolites may therefore be of considerable significance as mediators of pathological physiological reactions elicited by various types of injuries such as irritation and inflammation to pulmonary tissues. Studies were undertaken to investigate the metabolism of arachidonic acid by prostaglandin synthetase and the binding of the metabolites to tissue macromolecules. This reaction may be involved in mediating tissue damage in organs rich in prostaglandin synthetase. Also investigated was the formation of thromboxane A_2 from prostaglandin endoperoxide PGH_2 by thromboxan synthetase. Among other tissues, this enzyme is present in platelets and lung. Thromboxanes are thought to play an important role in platelet aggregation.

Prostaglandins are degraded by intracellular enzymes. The transport mechanism of prostaglandin is therefore an essential aspect of prostaglandin metabolism. The structural requirements of the PG molecule for transport were investigated and the effects of inhaled oxidant gases on its transport and metabolism were studied.

Other studies are concerned with the co-oxygenation of xenobiotics by the enzymes involved in PG metabolism. It was found that the carcinogen benzo(a)-pyrene is oxidized during the conversion of arachidonic acid to prostaglandins, resulting in formation of quinones. The possible consequences of this alternate pathway of BaP metabolism (alternate to the NADPH dependent cytochrome P-450 system) are being explored. Studies are underway in collaboration with the environmental carcinogenesis group to determine the significance of these findings in terms of mutagenesis and carcinogenesis.

BIOCHEMICAL PATHOLOGY

The major research efforts of this group are concerned with the biochemistry of the acellular lining layer of the terminal airways in health and disease. The importance of the acellular lining layer of the alveoli for normal function of the lung has been recognized for some years. Most of the investigations have been concerned with the surfactant activity of the phospholipid components.

Much less attention has been given to the protein constituents of the acellular, alveolar lining. Presently, the pulmonary pathology group's efforts are concerned mostly with the characterization of the enzyme activities of the lining layer in the adult and the identification of the origin of the enzymes. These investigations are being extended to include studies of the enzyme content of fetal lungs and amniotic fluid with the hope of identifying biochemical markers for lung maturation. Other studies are concerned with the biochemical analysis of alveolar secretions from patients with alveolar proteinosis. These experiments are expected to contribute to an understanding of the nature and pathogenesis of this disease. Similar studies on lungs from subjects exposed to noxious air contaminants will be carried out to search for sensitive enzymic markers of early lung injury.

CELL BIOLOGY

The pulmonary cell biology group is a new effort started in the middle of FY 78. Its main purpose is to provide basic information on the life cycle, and function of various cell types in the respiratory tract. At a later date, this will extend to studies of injury and recovery of specific cell types to gain a better understanding of the histopathological responses seen after toxic insults.

Initially, emphasis will be placed on epithelial cells, primarily of the conducting airways. The main approach used is tissue culture.

The difficulty in obtaining adequate information on the lifecycle and function of different cell types in the lung stems from the fact that the lung contains approximately 40 different cell types. This has been a severe obstacle to many biochemical studies. At present, most separation techniques are inadequate to provide cells of any one type in quantities sufficient for biochemical studies. New experimental approaches need to be developed to study individual cell types as well as the interaction of selected cell types.

Methods will be developed for the isolation of various cell populations. Since most epithelial cells, when grown in vitro rapidly dedifferentiate (or fail to differentiate) we anticipate, that a major long-term effort will be devoted to discover culture conditions, conducive to normal differentiation of respiratory tract epithelial cells. This group is expected to be also a major resource to other groups in the pulmonary branch.

ENVIRONMENTAL CARCINOGENESIS

This research effort, part of which is carried out by NIEHS staff located at the Oak Ridge National Laboratory, is concerned with selected problems of environmental carcinogenesis in two organ systems that are particularly prone to environmental insults, namely the lung and the skin. A great body of circumstantial evidence suggests that the induction of cancer in humans is in most cases a complex event, involving, besides the exposure to a carcinogen, a number of crucial interactions of host and environmental factors: (genetically determined) susceptibility, nutrition, age, exposure to various cofactors such as co-carcinogens and promoters etc. Much of the research effort carried out by the

carcinogenesis group is concerned with such interactions which increase the carcinogenic risk. Particularly in the respiratory tract, it has so far been difficult to study such problems adequately because of the lack of suited experimental systems. A number of new in vivo and in vitro models are, therefore, being developed and applied to carry out such co-factor studies.

Another area of research is concerned with the identification characterization of various stages of tumor development in epithelial tissues. It is well documented in humans and animals that a variety of epithelial alterations occur subsequent to carcinogen exposure and prior to development of neoplasia, which are presumed to be "preneoplastic lesions" (i.e. the progenitors of cancer). Detailed studies on the tissue and cell level are conducted to develop objective and quantitative criteria for the stages of neoplastic progression. This might ultimately enable us to identify those individuals early, who are highly susceptible to carcinogens and have an increased risk to develop cancer. The studies will also be useful to design methods for preventing the progression of preneoplastic cell populations to neoplasia.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80033-02 LPFT
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Synthesis of Polypeptide Hormones and Prohormones by Normal and Neoplastic Lung Epithelium		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Other:	R. P. DiAugustine L. H. Lazarus I. Linnoila A. Ghosh M. Khan P. Nettesheim M. L. Williams	Head, Endocrinology Group Research Chemist Visiting Associate IPA Visiting Fellow Chief, LPFT Technician, Biology Division Oak Ridge National Laboratory
		LPFT NIEHS LPFT NIEHS LPFT NIEHS LPFT NIEHS LPFT NIEHS LPFT NIEHS LPFT NIEHS
COOPERATING UNITS (if any) Pulmonary Carcinogenesis Program, Biology Division, Oak Ridge National Laboratory		
LAB/BRANCH Laboratory of Pulmonary Function and Toxicology		
SECTION Endocrinology Group		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 4.5	PROFESSIONAL: 4.0	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The lungs of mammals are known to contain <u>endocrine cells</u> and <u>neuroepithelial bodies</u> in the pulmonary epithelium. Neuroendocrine-type granules have been previously identified in both of these structures. Using immunocytochemical techniques we are attempting to identify known <u>polypeptide</u> hormones in normal and neoplastic lung epithelium of various species. Although no <u>adrenocorticotrophic hormone</u> (ACTH) was identified in normal lungs, this hormone was identified in cells obtained from the lungs of Syrian golden hamsters treated with <u>diethylnitrosamine</u>. These cells were also argyrophilic and contained numerous osmophilic bodies. Thus, these cells appear to be similar to those observed in human lung <u>small cell carcinomas</u>, which are known to contain several pituitary polypeptide hormones. Extracts of rodent lung <u>squamous cell carcinoma</u> induced by intratracheal injections of <u>3-methylcholanthrene</u> or localized exposure of <u>benz(a)pyrene</u> to tracheal transplants were found to contain <u>ACTH</u> and <u>proACTH</u> immunolike reactivity. Since proACTH has been found in the plasma of patients with squamous cell carcinoma, efforts are being continued to purify this protein from bovine pituitary glands.		

PROJECT DESCRIPTION

METHODS EMPLOYED: General histological procedures were used for fixation and staining of tissue sections. The immunoperoxidase bridge method was used for the immunocytochemical localization of polypeptide hormones. Other methods employed were radioimmunoassay, thin-layer and column chromatography and sodium dodecyl sulfate gel electrophoresis.

MAJOR FINDINGS AND PROPOSED COURSE: Previous studies have shown that increased levels of various polypeptide hormones have occurred in the plasma of patients with lung and other neoplasms. In some patients, generally those with lung small cell carcinoma, endocrine abnormalities such as Cushing's syndrome were evident. These findings have been attributed to ectopic synthesis of polypeptide hormones by various neoplasms.

In this investigation we have examined the capacity of various chemically-induced lung carcinomas to produce adrenocorticotrophic hormone and related polypeptides.

1. Chemically-induced squamous cell carcinomas. Four transplantable rodent lung squamous cell carcinomas were obtained through the Pulmonary Carcinogenesis Program at Oak Ridge National Laboratories. Two of these tumors were induced by intratracheal administration of 3-methylcholanthrene. The other two tumors were induced by localized exposure of benz(a)pyrene to tracheal transplants.

Extracts of these tumors contained 10-20 ng/gram wet weight of ACTH like immunoreactivity using a radioimmunoassay directed against the mid-sequence of ACTH. Dilutions of each extract yielded curves parallel to those obtained with the standard ACTH. The levels of ACTH in these rodent tumors are similar to those previously reported for human lung squamous cell carcinomas. Gel filtration of the extracts on columns of Sepharose CL-6B in the presence of 4M urea revealed a large molecular form of ACTH (>20,000 daltons) and a smaller form with an elution volume identical with that of ^{125}I -(1-39)ACTH.

The Grimelius silver staining method was used to identify any potential endocrine-like or enterochromaffin cells in these tumors. So far, examination of sections of one tumor showed that a small number of silver-positive cells were present suggesting that there is a heterogeneous population of cells. Using immunohistochemical methods, serial sections of the tumors will be examined for ACTH like immunoreactivity. It is possible that the low amount of ACTH present in lung squamous cell carcinomas can be accounted for by a smaller population of neoplastic endocrine cells.

2. Diethylnitrosamine-induced lung tumors. In an attempt to develop a cell line with similar morphological and growth properties to human small cell carcinoma, Syrian golden hamsters were treated with diethylnitrosamine (DEN). DEN has been previously reported to stimulate proliferation of lung epithelial cells which contain neuroendocrine-like granules. The lung epithelium of treated animals was examined for immunoreactive ACTH using radioimmunoassay

and immunocytochemical methods. The results from one trial revealed that ACTH-positive cells were induced in hamsters with DEN and it appears that these cells might survive in culture conditions and retain their capacity to be stained with silver and synthesize ACTH. No cells could be identified in the lung epithelium of normal hamsters which were immunopositive for ACTH. The possibility for low-level synthesis of pituitary hormones in normal epithelium will be considered.

3. Argyrophilic cells of the normal pulmonary epithelium. In addition to neuroepithelial bodies of the bronchioles or alveoli, it is well known that nearly all mammalian lungs contain epithelial cells which have neuroendocrine-like dense core vesicles. These cells are generally argyrophilic and demonstrate characteristic amine fluorescence following administration of precursors such as L-dopa. In the present study, argyrophilic cells have been identified in the pulmonary epithelium of the rabbit and guinea pig. A survey is being made using antisera to at least twenty different known polypeptide hormones as part of an effort to identify the specific function of these cells. Our routine studies employ the trachea of the guinea pig where these cells are regularly observed at about one percent of the total cell population. Other efforts are being made to determine (1) the homogeneity and ultrastructure of the argyrophilic cells, (2) their association or proximity to nerves, capillaries and other cells (3) fluorescent properties following administration of precursors to biogenic amines and (4) the hormonal and morphological properties and optimal growth conditions of these cells in vitro or in vivo.

4. Purification of the prohormone of ACTH. A large molecular weight form of ACTH has been identified as the common precursor to ACTH and β -lipotropin, a polypeptide known to contain and elaborate the sequence of the opiate-like peptide [Met⁵]-enkephalin. Using ion exchange, gel filtration and hydroxyapatite column chromatography, a protein has been purified from frozen bovine pituitary glands. The protein prohormone crossreacted with antisera to ACTH and (39-45) β -lipotropin, had a molecular weight of approximately 3×10^4 daltons and exhibited marked charge heterogeneity on ion exchange columns. Efforts are being made to obtain at least one nanomole of this protein for amino acid analyses and partial sequence studies. These data should allow antisera to be raised against specific sequences of the prohormone. Sensitive radioimmunoassays should be able to be developed for use in the tumor studies given above or for pituitary function tests.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Polypeptide hormones secreted by endocrine organs are known to have specific physiological effects at very low serum concentrations. Numerous clinical disorders are known to be due to abnormal levels of endocrine secretions or complementary receptors. Identification of polypeptide hormones and proteins produced by normal lung epithelial cells is important for understanding the factors which act to regulate selective lung functions. Identification and measurement of these specific peptides could be important in the assessment of damage induced by chemicals such as environmental carcinogens. Hypersecretion of cell-specific or tumor-specific polypeptides or proteins could

serve in the detection of lung preneoplasia or neoplasia on in the assessment of chemotherapeutic treatment of this disease.

PUBLICATIONS

Linnoila, R. I., DiAugustine, R. P., Miller, R. J., Chang, K. J. and Cuatrecasas, P.: An Immunohistochemical and Radioimmunological Study of the Distribution of [Met⁵]- and [Leu⁵]- Enkephalin in the Gastrointestinal Tract. In Press. Neuroscience.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25005-01 LPFT
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) The Binding and Release of Bioactive Polypeptides and Amines by Mast Cells		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: L. H. Lazarus Other: R. P. DiAugustine I. Linnoila M. Khan A. Ghosh R. Wu	Research Chemist Head, Endocrinology Group Visiting Fellow Visiting Fellow IPA IPA	LPFT NIEHS LPFT NIEHS LPFT NIEHS LPFT NIEHS LPFT NIEHS LPFT NIEHS
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Pulmonary Function and Toxicology		
SECTION Endocrinology Group		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 2.5	PROFESSIONAL: 2.5	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Mast cells are widely distributed in mammalian tissues but are most abundant in connective tissues. Special consideration is being given to the role of mast cells in the lung where these cells are mostly located beneath the epithelium in the trachea and bronchi, with some also present in the epithelium and mucus lining. Mast cells are known to contain bioactive amines such as histamine and serotonin and to bind the polypeptide neurotensin. This investigation focuses on: (1) the binding of other known polypeptides to specific receptors on mast cells (2) the release of humoral factors from mast cells in the presence of various polypeptide hormones and (3) the utilization of a masto-cytoma cell line to study the binding, processing and release of various bioactive polypeptides. Studies in this laboratory of a mouse mastocytoma have shown that this tumor is capable of synthesizing numerous polypeptide hormones heretofore not identified in mast cells. This tumor, therefore, appears to have the capacity of ectopic synthesis of polypeptide hormones similar to that previously demonstrated in various human tumors.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Mast cells (MC) are obtained from exsanguinated rats and mice by lavage of the peritoneal cavity with balanced salt solution. Mastocytoma cells are propagated in mice by intraperitoneal inoculations and collected by drainage of the peritoneal cavity. A cell line from the mastocytoma will be established in order to facilitate studies of the processing of specific polypeptides.

Iodination of peptide hormones is performed by the lactoperoxidase-glucose oxidase or the chloramine technique. The iodinated peptides are examined for retention of biological activity. In general, binding is performed at 4°C and the bound peptide-cell membranes retained on a filter or separated by centrifugation. Extraction of polypeptide hormones from MC is accomplished with mineral acids, organic solvents or with a denaturant such as urea or guanidine. Quantitation of peptide hormones is routinely made by radio-immunoassay or by assessment of biological activity, e.g., measurement of blood pressure changes or contractions of isolated smooth muscle preparations.

The processing and secretion by specific polypeptides will be studied by incorporation of labelled amino acids in pulse labelling/chase experiments *in vitro* or *in vivo*. Subsequent procedures will employ such methods as specific hormone antibody precipitation, sodium dodecyl sulfate polyacrylamide gel electrophoresis and gel filtration column chromatography. Unknown peptides in MC or mastocytoma will be isolated and characterized using ion exchange and gel filtration column chromatography, amino acid analysis, peptide mapping and sequencing by established procedures. Histamine and serotonin concentrations will be determined fluorometrically by using previously published procedures.

MAJOR FINDINGS AND PROPOSED COURSE: (1) Binding of polypeptide hormones: Although neurotensin reversibly binds to rat MC with a high degree of stereospecificity, no binding could be detected with the mouse mastocytoma. [Tyr⁸]-substance P also binds reversibly to MC, and not to mastocytoma. In contrast to neurotensin, substance P caused a marked release of histamine from the mastocytoma. It is possible that neurotensin can trigger the release of some other biologically active amine such as serotonin, or a polypeptide. Bradykinin will also be examined for its capacity to bind or effect release of humoral substances from MC and mastocytoma.

(2) Release of bioactive amines and polypeptides: The release of histamine from rat MC and mouse mastocytoma by peptide hormones is a specific process. Substance P and bradykinin are nearly as effective releasing agents as 48/80, whereas neurotensin and [Tyr⁸]-bombesin, an amphibian peptide with sequence homology to substance P, were ineffective as releasers. Dibutyl cyclic AMP and dexamethasone were also inactive as releasers of histamine from the mastocytoma. Numerous known peptides and their analogs will be analyzed for their capacity to release histamine and serotonin. Recently, other workers have shown that 48/80 was capable of releasing vasoactive intestinal polypeptide from rat MC.

These studies will be coordinated with other projects in this and other laboratories designed to identify polypeptides in nerves and cells of the mammalian lung. Such local peptides might be candidates for initiating responses of MC or vice versa.

(3) Synthesis and processing of polypeptide hormone: By using radioimmunoassay techniques and antisera developed in this laboratory, the presence of several polypeptide hormones have been detected in extracts of mastocytoma. Parallel displacement curves were obtained when extracts of mastocytoma were compared with those of known standards. Bovine (39-45) β -lipotropin, neurotensin, ACTH and substance P occur in nanogram quantities per ml of an extract of approximately 500 mg (wet wt) of cell mass. It is interesting to note that the detection of mouse β -LPH by the bovine sequence-specific antiserum might indicate sequence homology at the antibody recognition site. This antiserum specific to the (39-45) β -LPH bovine sequence does not significantly crossreact (<0.1%) with porcine or human β -MSH and α -MSH (melanocyte stimulating hormone). Porcine and human (39-45) β -LPH differ from bovine (39-45) β -LPH by one amino acid. Studies are in progress to determine by filtration chromatography and by acrylamide electrophoresis the approximate molecular weights of the peptides and their apparent precursors in mastocytoma.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It is well documented that the principal site of histamine storage is the MC, or in the case of blood, the basophil. MC in the pulmonary epithelium or submucosa release histamine during immune reactions occurring, for instance, with airborne antigens. Contraction of bronchiolar muscle and constriction of pulmonary arteries are some of the known pharmacological effects of histamine. Specific tetrapeptides obtained from mast cells have been shown by other workers to serve in the chemotaxis of eosinophils. There might be numerous potential responses of the MC in immune reactions in the lung. These responses could be regulated through specific cell surface receptors of MC with the subsequent release of various humoral factors. Understanding the many biological properties of the MC should provide a rationale for understanding some of the causal factors involved in allergic or related environmentally-induced pulmonary responses or other diseases.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25006-01 LPFT														
PERIOD COVERED October 1, 1977 through September 30, 1978																
TITLE OF PROJECT (80 characters or less) Studies on Thromboxane Synthetase																
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">M. W. Anderson</td> <td style="width: 35%;">Mathematician</td> <td style="width: 15%;">LPFT NIEHS</td> </tr> <tr> <td rowspan="3">Others:</td> <td>T. E. Eling</td> <td>Head, Prostaglandin Group</td> <td>LPFT NIEHS</td> </tr> <tr> <td>D. J. Crutchley</td> <td>Visiting Fellow</td> <td>LPFT NIEHS</td> </tr> <tr> <td>B. E. Tainer</td> <td>Biologist</td> <td>LPFT NIEHS</td> </tr> </table>			PI:	M. W. Anderson	Mathematician	LPFT NIEHS	Others:	T. E. Eling	Head, Prostaglandin Group	LPFT NIEHS	D. J. Crutchley	Visiting Fellow	LPFT NIEHS	B. E. Tainer	Biologist	LPFT NIEHS
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	B. E. Tainer	Biologist	LPFT NIEHS													
COOPERATING UNITS (if any) None																
LAB/BRANCH Laboratory of Pulmonary Function and Toxicology																
SECTION Prostaglandin Group																
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">TOTAL MANYEARS:</td> <td style="width: 33%;">PROFESSIONAL:</td> <td style="width: 33%;">OTHER:</td> </tr> <tr> <td style="text-align: center;">0.7</td> <td style="text-align: center;">0.4</td> <td style="text-align: center;">0.3</td> </tr> </table>			TOTAL MANYEARS:	PROFESSIONAL:	OTHER:	0.7	0.4	0.3								
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SUMMARY OF WORK (200 words or less - underline keywords) The unstable bioregulator <u>thromboxane A₂</u> (TXA ₂) is generated from the <u>prostaglandin (PG) endoperoxide PGH₂</u> by an enzyme called <u>thromboxane synthetase</u> . <u>Thromboxane synthetase</u> is present in many tissues including <u>blood platelets, lung, spleen and brain</u> . The formation of TXA ₂ and the C-17 hydroxy fatty acid HHT from PGH ₂ has been investigated, with emphasis on kinetics and inhibitors. It has been shown that HHT (12-Hydroxy-5,8,11-heptadecenoic acid) is not a breakdown product of TXA ₂ but that TXA ₂ breaks down exclusively into thromboxane B ₂ . Our results also show that thromboxane synthetase is not an isomerase but rather a bimolecular reaction is involved in the formation of TXA ₂ and perhaps HHT.																

PROJECT DESCRIPTION

METHODS EMPLOYED: Ram seminal vesicle microsomes were used to biosynthesize (14C)-PGH₂ from (14C)-arachidonic acid (AA). A washed human platelet preparation and human platelet microsomes were prepared from expired platelets obtained from the Red Cross. The conversion of PGH₂ and AA to TXA₂ and its stable endproduct thromboxane B₂ (TXB₂) was determined as a function of time of incubation and substrate concentration. Rates of formation of TXA₂ were determined as a function of PGH₂ concentration. TXA₂ concentrations were determined by converting free TXA₂ to O-methyl-TXB₂. PGH₂ concentrations were determined by reducing free PGH₂ to PGF₂ with triphenyl phosphine. The concentration of the hydroxy fatty acid 12L-hydroxy-5,8,10-heptadecatrienoic acid (HHT) was also determined in most of the studies.

MAJOR FINDINGS AND PROPOSED COURSE: Our data and reports in the literature show that TXB₂ and HHT are produced in approximately the same amounts when AA or PGH₂ are incubated with thromboxane synthesizing tissues. Inhibition of thromboxane synthetase appeared to inhibit the production of both TXB₂ and HHT to the same extent. One possible explanation of these observations is that TXA₂ could break down into either TXB₂ or HHT. However, our results explicitly show that HHT is not a breakdown product of TXA₂ but rather TXA₂ decomposes exclusively into TXB₂.

A model for the enzymatic generation of TXB₂ and HHT from PGH₂ has been proposed which is consistent with the above observation. It was suggested that an intermediate formed from PGH₂ can rearrange into either TXA₂ or HHT and malondialdehyde. This model is mechanistically consistent with the claim that thromboxane synthetase is an isomerase since one molecule of PGH₂ rearranges into either TXA₂ or HHT and malondialdehyde. However, our enzyme kinetic results are not consistent with this model. The rate of formation of TXB₂ is clearly dependent upon [PGH₂]² and thus a bimolecular reaction is involved in the formation of TXB₂. A reaction mechanism in which one molecule each of TXA₂ and HHT are produced simultaneously from two molecules of PGH₂ is consistent with our kinetic results as well as the above-mentioned observations.

The proposed course is as follows: Further investigate the proposal that TXA₂ and HHT are simultaneously generated from two molecules of PGH₂ by thromboxane synthetase.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Thromboxane synthetase is present in many tissues including lung, blood platelets, spleen and brain. Thromboxanes have very potent effects on smooth muscle including that of lung airways. They have been implicated as primary mediators in platelet aggregation. A variety of stimuli can release significant amounts of thromboxanes from the lung. Since prostaglandins and thromboxanes are not stored in tissues, understanding the factors that control and regulate

their biosynthesis is most important in understanding the physiological role of these bioactive substances.

PUBLICATIONS

Anderson, M. W., Crutchley, D. J., Tainer, B. E., and Eling, T. E.: Kinetic Studies on the Conversion of Prostaglandin Endoperoxide PGH_2 by Thromboxane Synthetase. Submitted to Biochem. Biophys. Res. Commun.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80Q08-04 LPFT																
PERIOD COVERED October 1, 1977 to September 30, 1978																		
TITLE OF PROJECT (80 characters or less) Studies on the Transport and Metabolism of Prostaglandins [PGs] in Lung																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 30%;">T. E. Eling</td> <td style="width: 40%;">Head, Prostaglandin Group</td> <td style="width: 20%;">LPFT NIEHS</td> </tr> <tr> <td>Other:</td> <td>M. W. Anderson</td> <td>Mathematician</td> <td>LPFT NIEHS</td> </tr> <tr> <td></td> <td>A. Chaudhari</td> <td>Visiting Fellow</td> <td>LPFT NIEHS</td> </tr> <tr> <td></td> <td>H. J. Hawkins</td> <td>Biologist</td> <td>LPFT NIEHS</td> </tr> </table>			PI:	T. E. Eling	Head, Prostaglandin Group	LPFT NIEHS	Other:	M. W. Anderson	Mathematician	LPFT NIEHS		A. Chaudhari	Visiting Fellow	LPFT NIEHS		H. J. Hawkins	Biologist	LPFT NIEHS
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	H. J. Hawkins	Biologist	LPFT NIEHS															
COOPERATING UNITS (if any) Inhalation Toxicology Section, Environmental Biology and Chemistry Branch																		
LAB/BRANCH Laboratory of Pulmonary Function and Toxicology																		
SECTION Prostaglandin Group																		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																		
TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.4	OTHER: 0.5																
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																		
SUMMARY OF WORK (200 words or less - underline keywords) It is the long-range goal of this project to study the inactivation of <u>prostaglandins</u> by the lungs and other tissues. The effects of environmental agents on the <u>pulmonary transport</u> and metabolism of <u>prostaglandins</u> is being investigated, and these effects are being related to <u>pulmonary toxicity</u> and <u>damage</u> caused by the environmental agents. Topics of current interest are the <u>structural requirements</u> of a prostaglandin needed for transport to intracellular spaces, the effect of <u>oxidant gases</u> on transport, and the potentiation of <u>inflammatory reaction</u> by inhibition of <u>pulmonary inactivation</u> of prostaglandins.																		

PROJECT DESCRIPTION

METHODS EMPLOYED: An isolated perfused rat, guinea pig, or rabbit lung was used to examine the uptake, metabolism, and efflux of prostaglandins (PGs) and their metabolites from lung tissue. The isolated perfused lung was designed to permit infusion of a constant concentration of PGs and perfusion with drug-free perfusate. PG metabolites were isolated from the perfusate by extraction and separated by thin-layer chromatography techniques. The uni-directional flux of PG into the lung was measured by extrapolation of the net uptake velocity of PG to zero time.

MAJOR FINDINGS AND PROPOSED COURSE: PGs are accumulated into lung tissue by a carrier-mediated process and are subsequently degraded by pulmonary enzymes to either 15-keto PG or 15-keto-13,14-dihydro PG. The uptake, metabolism, and subsequent release of parent PG and metabolites are remarkably efficient and rapid.

Structural activity studies were done to determine the critical moieties of the PG essential for transport into lung tissue. The following PGs were substrates for the pulmonary transport system: PGE₁, PGE₂, PGF_{1α}, PGD_{1α}, PGD₂, 15(S) methyl-PGF_{2α}, and PGF_{2α}. PGA₁, PGB₁, 15-keto PGF_{2α}, 15-(R) methyl PGF_{2α}, 15-keto PGE₁, 15-keto 13,14-dihydro PGE₁, 13,14-dihydro PGE₁, 13,14-dihydro PGF_{2α}, the methyl esters of PGF_{1α}, PGE₁, PGF_{2α}, and PGE₂, 15 epi-PGE₂ and 15 epi-PGF_{2α} were either poor substrates or not substrates for the pulmonary transport system. From these studies we have concluded that there are three critical portions of the PG molecules necessary for transport into lung tissue:

- (1) acid group at C-1
- (2) oxygen function, particularly a hydroxyl group at C-1
- (3) a hydroxyl group at C-15 in the S configuration.

Small changes in the geometric relationship of these three portions can greatly alter the lung's affinity to transport and metabolize a PG. For example, conversion of the 15 hydroxyl from an S to R configuration greatly reduces transport. Although we could not elucidate what is responsible for the substrate specificity, it is possible that the critical regions are necessary for the binding of a PG to a carrier molecule.

Recently, an additional PG, called PGI₂, was discovered by Vane and his co-workers. PGI₂ was shown to be a potent dilator of blood vessels and an inhibitor of platelet aggregation. It was proposed that a balance between PGI₂ and thromboxane A₂ could be of importance in cardio-vascular diseases. PGI₂ contains an additional ring formed between the carbon 9 oxygen and carbon 5 on the α side chain. This ring opens in aqueous solution resulting in the formation of 6-oxo PGF_{1α}. Although PGI₂ contains the 3 critical moieties required for transport, the α chain and thus the acid group have a different geometric configuration compared to the rest of the PG molecule. PGI₂ was rapidly degraded by pulmonary enzymes (*in vitro*) and appeared to be an excellent substrate for these enzymes. 6-oxo PGF_{1α} was also metabolized but was a poor substrate. However, on passage through the isolated perfused rat lung,

neither PGI_2 nor 6-oxo $\text{PGF}_{1\alpha}$ was metabolized. This serves as an additional example of the importance of the transport system in determining the pulmonary metabolism of PG. In contrast to other classical PG, pulmonary metabolism appears not to be the critical determinant of the in vivo half-life of PGI_2 .

We have also investigated the metabolism of PG by glutathione-S-aryl transferase. Previous studies have shown that PGA_1 is metabolized to water-soluble glutathione conjugates. We have shown that the 15-keto $\text{PGF}_{2\alpha}$ is also converted to glutathione conjugates by the enzyme glutathione-S-aryl transferase. Activity is highest in liver followed by kidney and lung. The presence of the 13-14 double bond is essential for conjugation and indicates that an α - β -unsaturated keto is the critical portion of the PG molecule required for conjugation. Since many 15-keto PGs have biological activity, conjugation by glutathione may be a new and important detoxification system for PG.

We have studied the effect of exposure of rats and guinea pigs to 100% O_2 on the pulmonary inactivation system. Exposure to 100% O_2 significantly depresses the enzyme system, prostaglandin dehydrogenase (PGDH), without effect on the transport system. NAD^+ , NADP^+ , PGDH succinic dehydrogenase, but not glucose-6- PO_4 dehydrogenase, were inhibited by exposure to O_2 . Kinetic studies indicate an uncompetitive inhibition of PGDH on exposure to O_2 which is consistent with the hypothesis that an inhibitor was generated by the exposure.

Exposure of rats to NO_2 first increased, then decreased the PGDH system. PGDH depressed by exposure to 100% O_2 recovered to normal enzyme levels 5 days after the animals were removed from the exposure to 100% O_2 .

The proposed course is as follows: 1) To examine the effect of exposure of rats to airborne pollutants on PG detoxification systems; 2) To use isolated cell systems to examine the mechanisms for the transport of PG into and out of cells and apply the information obtained from this study to the intact lung system; 3) To examine the effect of exposure of animals to pollutants on the inflammatory release of vaso-active chemicals from the lung.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: PGs have a large diversity of physiological effects. Alterations in PG control of cellular events may be related to transport of PGs across cell membranes. The lung is an important site for the synthesis and metabolism of PGs; alterations in the PG transport and metabolism systems may be related to toxic effects of exposures to pollutants and induction of lung diseases.

PUBLICATIONS

Anderson, M. W. and Eling, T. E.: Prostaglandin removal and metabolism by isolated perfused rat lung. Prostaglandins 11: 645-677, 1976.

Eling, T. E. and Anderson, M. W.: Studies on the biosynthesis, metabolism, and transport of prostaglandins by the lung. Agents and Actions 6: 543-546, 1976.

Eling, T. E., Hawkins, H. J., and Anderson, M. W.: Structural requirements for and the effect of chemicals on the pulmonary inactivation of prostaglandins. Prostaglandins 14: 51-60, 1977.

Hawkins, H. J., Wilson, A. G. E., Anderson, M. W., and Eling, T. E.: Uptake and metabolism of prostaglandins by isolated perfused lung: Species comparisons and the role of plasma protein binding. Prostaglandins 14: 251-259, 1977.

Hawkins, H. J., Anderson, M. W. and Eling, T. E.: Studies on the passage of PGI_2 through the pulmonary circulation. Prostaglandins. Submitted.

Chaudhari, A., Eling, T. E. and Anderson, M. W.: Inhibition of prostaglandin metabolism by exposure of animals to oxygen or nitrogen dioxide. Biochem. J. Submitted.

Chaudhari, A., Anderson, M. W., and Eling, T. E.: Conjugation of 15-keto-prostaglandins by glutathione-S-transferase. Biochem. Biophys. Acta. Submitted.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80034-02 LPFT																				
PERIOD COVERED October 1, 1977 to September 30, 1978																						
TITLE OF PROJECT (80 characters or less) Covalent Binding of Arachidonate Metabolite(s) from Prostaglandin Synthesis to Tissue																						
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																						
<table style="width: 100%; border: none;"> <tr> <td style="width: 10%; vertical-align: top;">PI:</td> <td style="width: 40%;">M. W. Anderson</td> <td style="width: 30%;">Mathematician</td> <td style="width: 20%;">LPFT NIEHS</td> </tr> <tr> <td style="vertical-align: top;">Other:</td> <td>T. E. Eling</td> <td>Head, Prostaglandin Group</td> <td>LPFT NIEHS</td> </tr> <tr> <td></td> <td>H. J. Hawkins</td> <td>Biologist</td> <td>LPFT NIEHS</td> </tr> <tr> <td></td> <td>D. J. Crutchley</td> <td>Visiting Fellow</td> <td>LPFT NIEHS</td> </tr> <tr> <td></td> <td>B. E. Tainer</td> <td>Biologist</td> <td>LPFT NIEHS</td> </tr> </table>			PI:	M. W. Anderson	Mathematician	LPFT NIEHS	Other:	T. E. Eling	Head, Prostaglandin Group	LPFT NIEHS		H. J. Hawkins	Biologist	LPFT NIEHS		D. J. Crutchley	Visiting Fellow	LPFT NIEHS		B. E. Tainer	Biologist	LPFT NIEHS
PI:	M. W. Anderson	Mathematician	LPFT NIEHS																			
Other:	T. E. Eling	Head, Prostaglandin Group	LPFT NIEHS																			
	H. J. Hawkins	Biologist	LPFT NIEHS																			
	D. J. Crutchley	Visiting Fellow	LPFT NIEHS																			
	B. E. Tainer	Biologist	LPFT NIEHS																			
COOPERATING UNITS (if any) Environmental Biology and Chemistry Branch; Laboratory of Environmental Mutagenesis																						
LAB/BRANCH Laboratory of Pulmonary Function and Toxicology																						
SECTION Prostaglandin Group																						
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																						
TOTAL MANYEARS: 2.7	PROFESSIONAL: 1.9	OTHER: 0.8																				
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																						
SUMMARY OF WORK (200 words or less - underline keywords) The goal of this project is to determine the relationships between the <u>covalent binding</u> of arachidonic acid metabolites produced by <u>prostaglandin synthetase</u> to tissue <u>macromolecules</u> and <u>pulmonary toxicity</u>. We wish to study the mechanism responsible for this covalent binding, what controls this process, and if it is of biological significance. Emphasis is being placed on what metabolite(s) binds and what regulates the covalent binding.																						

PROJECT DESCRIPTION

METHODS EMPLOYED: Prostaglandin (PG) and thromboxane (TX) synthetase activity was measured in vitro using the microsomal protein from a variety of tissues and organs as an enzyme source. ^{14}C -Arachidonic acid (AA) or prostaglandin endoperoxides were incubated at 37°C for various times and under several conditions. After incubation, the PG and TX were removed by solvent extraction, separated by thin-layer chromatography, and estimated by liquid scintillation techniques. Protein was precipitated by TCA and exhaustively washed with solvents. Amount of protein associated radioactivity was determined by liquid scintillation counting.

MAJOR FINDINGS AND PROPOSED COURSE: Incubation of (1- ^{14}C)-arachidonic acid with a preparation of guinea pig lung microsomes led to the formation of protein associated radioactivity which could not be dissociated by the exhaustive solvent extraction procedures employed. The protein-associated material formed was not dissociated by digestion with guanidine-HCl followed by Sephadex chromatography, or by refluxing for 60 min. with methanol. Indomethacin inhibited the formation of prostaglandins and thromboxanes and also reduced the amount of the protein-associated radioactivity. The protein-associated radioactivity was dramatically reduced by the addition of glutathione to the incubation mixture although there was no effect on the prostaglandin and thromboxane metabolites of arachidonic acid. These results strongly suggest, although they do not conclusively prove, that the protein-associated material is bound covalently and that the covalently linked radioactivity is derived from the metabolism of arachidonic acid by prostaglandin synthetase.

The covalently linked radioactivity is also observed when (1- ^{14}C)-arachidonic acid is incubated with the microsomal fraction of other cyclooxygenase-containing tissues, including normal ram or bovine seminal vesicles, rabbit kidney, rat fundus and human platelets. The stable prostaglandin and thromboxane endproducts do not associate with protein. These results suggest that a common intermediate must be responsible for the covalently bound radioactivity. The prostaglandin endoperoxides, PGG_2 and PGH_2 , are common intermediates to all the prostaglandins and thromboxanes and are chemically reactive compounds.

The prostaglandin endoperoxides PGG_2 and PGH_2 do indeed bind covalently to the protein of microsomes. The rate of binding was shown to be directly proportional to the free endoperoxide concentration. The rate constant for the covalent binding of PGG_2 was approximately twice that for PGH_2 , presumably indicating that both the 9,11-endoperoxy and 15-hydroperoxy groups participate in the binding reaction. Furthermore, the covalent binding observed when (1- ^{14}C)-arachidonic acid was incubated with microsomal preparations can be attributed to the prostaglandin endoperoxides since the degree of binding at any time was directly related to the free endoperoxide concentration. The rate constant of binding derived from arachidonic acid approximated those of the purified endoperoxides.

The covalent binding of lipid peroxides to tissue macromolecules is not without precedent. The formation of adducts between protein and lipid peroxide generally proceeds via the formation of free radicals. Prostaglandin endoperoxides may covalently bind to protein by a similar type of mechanism. Such a mechanism is consistent with the finding that the binding of PGG_2 to protein was inhibited by diethyldithiocarbamate and by phenylbutazone. Diethyldithiocarbamate inhibits free radical propagation; phenylbutazone, which possesses an enolic hydrogen may also act as an antioxidant. Alternatively, the binding may proceed via interaction of the endoperoxides with nucleophilic sites on the protein. Electrophilic oxides produced from a variety of xenobiotics by the mixed-function oxidase system bind covalently to tissue macromolecules. Prostaglandin, endoperoxides which are electrophilic compounds, may bind similarly, although such a mechanism does not account for the ability of diethyldithiocarbamate to inhibit the binding of PGG_2 .

Since the generation of covalently bound material is dependent on free endoperoxide levels, the degree of binding observed in any one tissue will be mainly determined by the relative activity of the respective cyclooxygenase, and the nature of enzymes converting the endoperoxides to their respective endproducts. In general, we have observed that the degree of covalent binding, expressed as a percentage of total arachidonate metabolism, was lower in tissues that synthesize thromboxanes than in those that do not. This is probably a reflection of the relatively high activity of thromboxane synthetase, which would tend to reduce free endoperoxide levels in the tissues concerned.

Glutathione reduced covalent binding without significantly affecting total product formation from (1-14C)-arachidonic acid. This is in contrast to the action of indomethacin, which blocked cyclooxygenase and thereby reduced both covalent binding and total product formation. The inhibition of covalent binding by glutathione is probably complex. Incubation of guinea pig lung microsomes and (1-14C)-arachidonic acid in the presence of glutathione reduced covalently bound material and led to the formation of water-soluble glutathione adducts; however, the increase in water soluble metabolites did not account completely for the inhibition of covalent binding, suggesting that other mechanisms are involved. Glutathione is an absolute requirement for prostaglandin E isomerase, and stimulates the conversion of PGH_2 to PGE_2 . Such an action would reduce the formation of covalently bound material by reducing the available free endoperoxide concentration. A similar stimulation of end-product formation at the expense of covalent binding was observed in the effect of norepinephrine in rat fundus microsomes.

In addition to the covalently bound metabolites generated by the metabolism of AA, water soluble metabolites were also observed in each of the tissues examined. We had previously shown that these water soluble metabolites arise in part from the conjugation of GSH with an intermediate(s) in AA metabolism. Our present results are consistent with that observation. The amount of water soluble metabolites increased as the GSH concentration increased. It has been

previously shown that cysteine adds to lipid hydroperoxides. Perhaps the prostaglandin endoperoxides are forming adducts with GSH in a similar fashion. The exact nature and role of the water soluble metabolites require further investigation.

The proposed course is as follows: 1) Further examine the mechanism of covalent binding; 2) Further study the inhibition of covalent binding with antioxidants and with anti-inflammatory compounds which are not good inhibitors of the prostaglandin cyclooxygenase; 3) Assess the role of prostaglandin endoperoxides in promoting lipid peroxidation and to see if the covalent binding is an indication of lipid peroxidation; 4) Obtain an in vivo model to study covalent binding of prostaglandin endoperoxides.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Under normal conditions, the balance between generation and their conversion to endproducts is probably finely controlled, and little free endoperoxide occurs. However, under conditions which cause excess arachidonate oxidation, or reduction in the activity of enzymes degrading the endoperoxides, sufficient accumulation of endoperoxides may occur to lead to significant covalent binding to protein. Prostaglandin endoperoxides have been detected in the effluent from sensitized guinea pig lungs following anaphylaxis and are released from platelets during aggregation by arachidonate. The role that the covalent binding of endoperoxides to tissue macromolecules has in physiological or pathological processes is unknown at present. However, carcinogenic and other pathological effects, such as liver and bronchiolar necrosis, have been associated with the covalent binding of the electrophilic oxides of chemicals to tissue macromolecules. The irritation or stimulation of tissues high in prostaglandin synthetase, such as the lung, by various chemicals, particles or environmental agents results in increased production of prostaglandin endoperoxides and thus possible higher levels of metabolites covalently bound to tissue macromolecules. The covalent binding of the endoperoxide to tissue macromolecules may initiate or be involved in the induction of tissue damage in a manner similar to toxicity produced by the covalent binding of exogenous electrophiles produced from various chemicals. Tissue injury in inflammation could, in part, result from the covalent binding of prostaglandin endoperoxides or to the promotion of lipid peroxidation by prostaglandin endoperoxides since elevated levels of prostaglandins are found in inflamed tissue. The potential role of the covalent binding of the endoperoxides to tissue macromolecules to initiate a toxic response or to sequester potentially toxic intermediates requires further investigation.

PUBLICATIONS

Eling, T. E., Wilson, A. G. E., Chaudhari, A., and Anderson, M. W.: Covalent binding of an intermediate(s) in prostaglandin biosynthesis to guinea pig lung microsomal protein. Life Sci. 21, pp. 245-252 (1977).

Anderson, M. W., Crutchley, D. J., Chaudhari, A., Wilson, A. G. E., and Eling, T. E.: Studies on the covalent binding of an intermediate(s) in prostaglandin biosynthesis to tissue macromolecules. Submitted to Biochim. Biophys. Acta.

Crutchley, D. J., Hawkins, H. J., Eling, T. E., and Anderson, M. W.: Covalent binding of prostaglandins G_2 and H_2 to ram seminal vesicle microsomes. Submitted to J. Biol. Chem.

Wilson, A. G. E., Kung, H. C., Anderson, M. W., and Eling, T. E.: Covalent binding of intermediates formed during the metabolism of arachidonic acid by human platelets. Submitted to Biochem. Biophys. Acta.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80035-02 LPFT
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Co-oxygenation of Xenobiotics by the Prostaglandin and Thromboxane Synthetase		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Other:	T. E. Eling M. W. Anderson K. Sivarajah H. J. Hawkins J. C. Barrett E. E. Sisskin	Head, Prostaglandin Group Mathematician Visiting Fellow Biologist Senior Staff Fellow Staff Fellow
		LPFT NIEHS LPFT NIEHS LPFT NIEHS LPFT NIEHS LPFT NIEHS LPFT NIEHS
COOPERATING UNITS (if any) Environmental Biology and Chemistry Branch		
LAB/BRANCH Laboratory of Pulmonary Function and Toxicology		
SECTION Prostaglandin Group		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.6	OTHER: 0.4
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) It is the long-range goal of this project to study the <u>co-oxygenation of xenobiotics</u> by prostaglandin synthetase, particularly the <u>pulmonary prostaglandin synthetase</u>. The generation of toxic intermediates, <u>carcinogens</u>, <u>mutagens</u>, etc., during the co-oxygenation will be investigated. Topics of current interest are the <u>co-oxygenation of benzopyrene</u> during the metabolism of arachidonic acid by <u>guinea pig lung</u>, <u>ram seminal vesicle</u> microsomes, and cells grown in tissue culture. BP was oxidized to quinones by PG synthetase. Electrophilic metabolites of BP were formed since BP was found covalently linked to protein and DNA. Significance of this metabolic activation in relation to cell transformation is being examined.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Microsomal preparations of various tissues, such as guinea pig lung and ram seminal vesicles, were used to examine the co-oxygenation of xenobiotics during prostaglandin synthesis. The benzopyrene metabolites were isolated from the incubation medium by extraction and by a preliminary separation by thin-layer chromatography techniques. Prostaglandin and thromboxane products were also isolated and quantitated. Tissue cultures are used to examine the interaction of BP electrophilic metabolites produced by PG synthetase and to determine if this interaction is related to cell transformation.

MAJOR FINDINGS AND PROPOSED COURSE: The carcinogen benzopyrene (BP) is oxidized during conversion of arachidonic acid (AA) to prostaglandins (PG) and thromboxanes (TX). The major BP metabolites were identified as quinones by TLC systems. In comparison, BP was oxidized by the NADPH-dependent P-450 system to mainly dihydrodiol and monohydroxylated BP metabolites. Although the total NADPH-dependent BP oxidation was 2-3X the AA-dependent oxidation, the amount of quinones formed by each system was similar. BP was oxidized by both the AA-dependent and NADPH-dependent systems to metabolites which were electrophilic as indicated by BP associated radioactivity remaining covalently linked to protein and DNA. Surprisingly, the percentage of total BP metabolites which were linked to tissue macromolecules was as high as 17% by the AA-dependent oxidation, compared to 5% by the NADPH-dependent oxidation. We and others have shown that AA-dependent BP oxidation was inhibited by anti-oxidants which are known inhibitors of BP-induced cell transformations. Oxophenbutazone is oxidized during PG synthesis to 4-hydroxy-oxyphenbutazone which does not subsequently further oxidize to quinone structure. Preliminary studies with oxyphenbutazone indicate that this chemical covalently links to tissue. Thus, we are proposing that AA-dependent BP oxidation proceeds via a free radical mechanism which generates a BP free radical which reacts with tissue macromolecules. The stable BP metabolites are quinones which arise from the auto-oxidation of 6-hydroxy BP. 7,12-Dimethyl benzantracene was also oxidized to electrophilic metabolites by PG synthesis. Our proposed course is as follows: 1) To further study the mechanism of AA-dependent BP oxidation and to determine the nature of electrophilic metabolites; 2) To study AA-dependent oxidation in tissue cultures, to determine that significant oxidation occurs in vivo and if BP metabolites are covalently bound to DNA and protein; 3) Using tissue cultures and in vitro mutagen test systems, to assess the significance of AA-dependent BP oxidation related to cell transformation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many xenobiotics are thought to exert their toxicity by means of reactive metabolites which are generated in vivo with very short half-lives. These reactive metabolites react with tissue macromolecules to produce toxicity such as carcinogenesis, mutagenesis, teratogenesis, etc. The prostaglandin synthetase system is found in most mammalian tissues and has particularly high levels

of activity in the lung and kidney. Moreover, arachidonic acid can be released from its phospholipid storage sites by various types of stimulation, for example, irritation of lung tissue by inhaled pollutants. The subsequent metabolism of arachidonic acid by prostaglandin synthetase and the simultaneous co-oxygenation of xenobiotics could produce toxic metabolites of xenobiotics.

PUBLICATIONS

Sivarajah, K., Anderson, M. W., and Eling, T. E.: Metabolism of Benzo(a) pyrene to Reactive Intermediate(s) via Prostaglandin Biosynthesis, Life Sci. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80023-05 LPFT
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) The Composition and Origins of the Acellular Lining Layer of the Lung		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: G. E. R. Hook J. W. Spalding Other: D. Nadeau D. Y. Bell	Senior Staff Fellow Biologist Visiting Fellow IPA	LPFT NIEHS LPFT NIEHS LPFT NIEHS LPFT NIEHS
COOPERATING UNITS (if any) Environmental Chemistry and Biology Branch		
LAB/BRANCH Laboratory of Pulmonary Function and Toxicology		
SECTION Biochemical Pathology		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.5	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The terminal airways of the lung are lined with an acellular layer of material which appears vital in the maintenance of normal pulmonary function. The composition and origins of the <u>acellular lining layer</u> are being investigated. Current attention has been directed towards: (1) the biosynthesis and <u>secretion</u> of <u>pulmonary</u> surfactant, and (2) the acellular lining layer as an actively metabolizing medium. The objectives of this investigation are as follows: (1) to determine the enzymatic and protein composition of the lung lining, (2) to determine the origin of these activities, and (3) to investigate compositional variations and biosynthesis of the lining under the influence of drugs, disease, and airborne pollutants.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Acellular lining layer material is obtained by lavaging the lungs via the trachea. Particulate materials are isolated from the lavage by isopycnic centrifugation on sucrose gradients. Lamellar bodies as found in alveolar Type II cells are isolated using differential centrifugation. Enzyme and protein analyses are carried out using polyacrylamide gel electrophoresis and isoelectric focusing.

MAJOR FINDINGS AND PROPOSED COURSE: The extracellular lining of the distal airways and alveoli is an enzymatically active medium containing a number of hydrolytic enzymes. The most active hydrolases present are α -mannosidase, β -N-acetylglucosaminidase and β -galactosidase. The hydrolases are components of the lamellar bodies of the alveolar type II cell and appear to be secreted into the alveoli along with pulmonary surfactant. The hydrolases are present in the acellular lining of the lungs of rabbits, rats, guinea pigs and humans.

Phospholipid exchange proteins have been identified in the cytosol of mammalian lung homogenates. These phospholipid exchange proteins can facilitate the exchange of phospholipids between different fractions of lung subcellular organelles, e.g., mitochondria and microsomes, and between these organelles and synthetic liposomes. Lung phospholipid exchange proteins also enhance the exchange of phospholipid between microsomes and lamellar inclusion bodies isolated from the lung.

Lamellar bodies isolated from the lungs of rabbits will be investigated for their hydrolases (usually considered to be of lysosomal origins) in an attempt to understand the relationship between lamellar bodies and lysosomes.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The composition of the acellular lining layer of the lung is complex consisting of enzymes, proteins, and lipids. The origins of these many components are obscure.

Our studies provide evidence that phospholipid transfer proteins may be involved in the biosynthesis of lamellar bodies and other subcellular organelles of the lung and that the Type II cell is involved in the secretion of not only phospholipids but hydrolytic enzymes as well.

The acellular lining layer of the terminal airways is vital for normal lung function. The influence of inhaled environmental toxicants on this layer is not known, but in many environmentally related pulmonary diseases (e.g., silico-alveolar proteinosis), the composition of the acellular lining layer appears to be altered. The involvement of the acellular layer in the progression or mediation of pulmonary disease is not known. Information relating to the composition and origins of the acellular lining layer and its variation following inhalation of toxicants is of immediate concern to occupational and environmental health.

PUBLICATIONS

Hook, G. E. R.: Extracellular hydrolases of the lung. Biochemistry 17, 520-528 (1978).

Hook, G. E. R. and DiAugustine, R. P.: Secretory cells of the peripheral pulmonary epithelium as targets for toxic agents. Environ. Health Perspect. 16, 147-156 (1976).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80029-02 LPFT																
PERIOD COVERED October 1, 1977 to September 30, 1978																		
TITLE OF PROJECT (80 characters or less) Investigations of Human Pulmonary Diseases																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">G. E. R. Hook</td> <td style="width: 35%;">Senior Staff Fellow</td> <td style="width: 15%;">LPFT NIEHS</td> </tr> <tr> <td></td> <td>D. Nadeau</td> <td>Visiting Fellow</td> <td>LPFT NIEHS</td> </tr> <tr> <td></td> <td>D. Y. Bell</td> <td>IPA</td> <td>LPFT NIEHS</td> </tr> <tr> <td>Other:</td> <td>S. E. Fabro</td> <td>Consultant</td> <td>LET NIEHS</td> </tr> </table>			PI:	G. E. R. Hook	Senior Staff Fellow	LPFT NIEHS		D. Nadeau	Visiting Fellow	LPFT NIEHS		D. Y. Bell	IPA	LPFT NIEHS	Other:	S. E. Fabro	Consultant	LET NIEHS
PI:	G. E. R. Hook	Senior Staff Fellow	LPFT NIEHS															
	D. Nadeau	Visiting Fellow	LPFT NIEHS															
	D. Y. Bell	IPA	LPFT NIEHS															
Other:	S. E. Fabro	Consultant	LET NIEHS															
COOPERATING UNITS (if any) Department of Medicine, Duke University; Environmental Biology and Chemistry Branch; Columbia Hospital for Women, Department of Gynecology and Obstetrics, George Washington University.																		
LAB/BRANCH Laboratory of Pulmonary Function and Toxicology																		
SECTION Biochemical Pathology																		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																		
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SUMMARY OF WORK (200 words or less - underline keywords) This project is concerned with the identification and characterization of components of the lung associated with pulmonary diseases which could provide information concerning the disease processes as well as function as diagnostic markers of pulmonary damage or disease. Current attention is being focused on (1) the enzyme components of <u>human amniotic fluid</u> which originates in the <u>fetal lung</u>; (2) the particulate materials, and soluble proteins present in the lungs of patients with <u>alveolar proteinosis</u>. The objectives are as follows: (1) to identify lung-specific enzymes and proteins which are released into serum or the pulmonary airways following the production of cellular damage by inhaled toxicants, (2) to investigate and characterize unusual enzymes, proteins, cells and particulate materials present in diseased lungs, and (3) to identify enzymes or proteins in human amniotic fluid which originate from the fetal lung and to investigate the use of such components to predict fetal lung maturity.																		

PROJECT DESCRIPTION

METHODS EMPLOYED: Broncho-alveolar lavage effluents from patients with pulmonary diseases are obtained from the Department of Medicine at Duke University. The isolation of particulate components from the lavage effluents involve differential and isopycnic centrifugation procedures.

Enzyme investigations involve the use of gel-filtration chromatography, polyacrylamide gel electrophoresis, isoelectric focusing on polyacrylamide gels, immunoelectrophoresis, and differential centrifugation. Amniotic fluid is obtained during Caesarian delivery of term mothers at the Columbia Hospital for Women in Washington, DC. Pulmonary lavage effluents from patients with alveolar proteinosis is obtained as a by-product of the therapy essential to the well-being of these patients. The lavage procedure involves instillation of isotonic saline into the left or right lung of the patient followed by drainage.

MAJOR FINDINGS AND PROPOSED COURSE: 1. Soluble alkaline phosphatase present in lavage effluents from the lungs of patients with pulmonary alveolar proteinosis has been purified and compared with the soluble alkaline phosphatase present in homogenates of nondiseased human lungs obtained at autopsy. The kinetic properties including pH optima, response to inhibitors such as aminoacids, Michaelis constant, and activation by magnesium ions appear similar. However, the molecular weights of the two enzymes following treatment with butanol differ (215,000 and 250,000 daltons for the disease-associated and normal enzymes, respectively).

2. Pulmonary Components in Amniotic Fluid. Fetal lung derived components have been sought in amniotic fluid obtained during Caesarean delivery of full-term infants. Phospholipid-rich lamellar body-like structures have been isolated from the amniotic fluid. Morphologically, these structures resemble lamellar bodies of the alveolar Type II cell and lamellar body derived structures often seen in the extra-cellular lining layer of adults' lungs. In normal humans lamellar bodies are found associated only with the lung, and consequently there is little doubt that the lamellar body-like structures found in amniotic fluid have arisen from the fetal lung. The lamellated structures have been isolated using isopycnic centrifugation on sucrose gradients. The isolated and purified structures are phospholipid rich (phospholipid: protein = 10:1). The two major phospholipids are phosphatidylcholine (80%) and phosphatidylglycerol (10%). The most abundant fatty acid present in the phosphatidylcholine is palmitate (85%).

3. Lavage effluents from the lungs of patients with pulmonary alveolar proteinosis were analyzed for soluble protein constituents. Antisera mono-specific for normal plasma components, C-reactive protein and secretory piece were used to investigate the presence of these proteins in lavage effluent, while two-dimensional polyacrylamide gel electrophoresis provided a comprehensive map of the major proteins present. The proteins of lung washings obtained from normal subjects and of patient and normal sera were similarly analyzed. Lavage

effluent from patients contain large quantities of soluble proteins (approximately 25 g/lung). Most of the proteins present (e.g., albumin, IgG, transferin, α_1 -antitrypsin) are similar to those found in normal lungs and serum. However, patients had in their lung lavage effluents two soluble proteins not found in their sera and not present in lung washings from normal subjects. It is not established whether these proteins are unique to pulmonary alveolar proteinosis, but they were not present in lavage effluents from two patients with bronchial asthma. Uniformly elevated levels of immunoglobulins in patient lavage effluent, abnormal immunoglobulin levels in patient serum, and the presence of C-reactive protein in four of five patient sera indicate involvement of the immune system in the disease process and suggest that an atypical hypersensitivity reaction may be the cause.

The materials present in lavage effluents from both diseased and normal human lungs will be further characterized. Disease associated enzymes and proteins will continue to be investigated.

The lamellar bodies present in human amniotic fluid will be characterized as to their protein content and animal models will be investigated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: 1) Many human pulmonary diseases have been described but unfortunately the diagnosis of these diseases is not usually made until the disease is well advanced. X-ray methods generally are not capable of detecting pulmonary diseases excepting in the advanced stages and even then are often incapable of distinguishing between diseases. Methods for the diagnosis of pulmonary disease and the detection of pulmonary damage in the earliest possible stages are needed most urgently.

The association of unusual proteins and a highly active alkaline phosphatase with the materials present in the pulmonary airways and alveoli of patients with alveolar proteinosis may prove useful in the diagnosis of this disease as well as provide information concerning the origins of the disease.

Our studies of the human amniotic fluid indicate that phospholipids secreted into the alveoli of the fetal lung are transferred to the amniotic fluid in the form of secreted lamella bodies. Reliable methods for the prediction of the respiratory distress syndrome may be devised based upon these observations since the lamellar bodies isolated from amniotic fluid are themselves direct measure of the ability of the fetal lung to secrete pulmonary surfactant. Absence of pulmonary surfactant is directly responsible for the respiratory distress syndrome.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25004-01 LPFT
PERIOD COVERED February 1, 1978 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Studies in Establishing Pulmonary Epithelial Cells in Culture		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Reen Wu IPA LPFT NIEHS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Pulmonary Function and Toxicology		
SECTION Pulmonary Cell Biology		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER:
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) More than 40 different types of cells have been identified in the lung, reflecting a diversity of metabolic activity. Advances in our understanding of the biochemical functions of the lung will depend on progress in <u>in vitro</u> culture studies. Appropriate conditions for culturing pulmonary epithelial cells are lacking. Therefore, the initial goal of the Pulmonary Cell Biology Group is to develop culture conditions for these epithelial cells. We have observed in a primary lung cell culture that the maintenance of epithelial cells in culture depends on medium conditioned by the mouse BalbC 3T3 cells. With this conditioned medium, the growth of fibroblastic cells is retarded, while epithelial cells grow slowly but steadily. More than 30 epithelial clones were selected. In the preliminary study, two clones, C05 and D07 differing in their clonal morphologies were recloned and further studied. Results have shown that both cells depend on the conditioned medium for plating at low cell density. Furthermore, C05 shows a dependence on the conditioned medium for growth. These results suggest that the conditioned medium can be used for the <u>clonal isolation</u> of different types of pulmonary epithelial cells and for the maintenance of these cells in culture.		

PROJECT DESCRIPTION

METHODS EMPLOYED: The medium used in this study was Dulbecco Modified Eagle's medium (DME) with the supplement of 10% fetal bovine serum (FBS). The so-called "conditioned medium" was prepared from confluent culture medium of mouse Balb C 3T3 cells. The lung from an adult female Albino rat was minced and digested by 0.1% collagenase (37°C, 30 min), and dispersed cells were directly plated into culture dishes.

MAJOR FINDINGS AND PROPOSED COURSE: The success in culturing different types of pulmonary epithelial cells in vitro has been very limited, despite progress in in vitro study of the alveolar type II cells. We reason that the so-called "mesenchymal factor" may be needed for the growth of lung epithelial cells. We have observed that the growth of the primary epithelial cell culture in the conditioned medium is slow but steady. However, the growth of fibroblastic cells in this primary culture is inhibited in the conditioned medium. This result suggests that the conditioned medium can limit the outgrowth of fibroblastic cells which are undesirable and a common problem during the clonal isolation of epithelial cells. More than 30 clones of epitheloid-like cells were selected. In the preliminary study, two clones, C05 and D07 differing in their clonal morphologies were further studied and recloned. Both cell lines have been kept in the conditioned medium for more than 6 months (about 25 passages), and they still maintain their distinctive appearances and growth characteristics. Results have shown that the conditioned medium increases the plating efficiency of these two epithelial cell lines, especially at low cell density. In addition, C05 cells show a dependence on the conditioned medium for growth. Without the conditioned medium, cell growth is decreased. Both cell lines show a 30 to 40 hrs doubling time in the conditioned medium. However, with the additional supplement of 1 to 5% FBS to the conditioned medium, the doubling time decreases to 20-24 hrs. This result suggests that some nutrients necessary for these epithelial cells are also depleted by 3T3 cells.

The origins and the cellular differentiated functions of these epithelial cells remain to be established. More importantly, we like to establish whether 3T3 cell-conditioned medium can be used to establish other pulmonary epithelial cells in culture. We have shown that the conditioned medium can be used for the clonal isolation of epithelial cells in culture.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Within the last decade, culture techniques have been developed that permit differentiated cells derived from normal tissues to retain specialized characteristics while undergoing a series of replicative cycles. The advantage of studying a specific cell type in culture is apparent: the number of variables to be controlled is markedly reduced. With more than 40 different types of cell having been identified in the lung, the importance to study these cells in vitro is quite apparent.

There is much evidence indicating the effect of secretory products on neighboring cells. The endodermal heritage of most pulmonary epithelial cells is likely to respond to the product(s) secreted by mesenchymal cells. Our results in growth stimulation and increase of plating efficiency of two epithelial clones by the 3T3 cell-conditioned medium should have relevance to in vivo situation. To extend this study further, it is possible to culture any types of pulmonary epithelial cells in vitro if the appropriate environment is well provided. The cell culture system will clearly provide further understanding of the nature of diverse functions in the lung.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25000-01 LPFT
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Cultured Epidermal Cells as a Model for Skin Carcinogenesis		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Other:	E. E. Siskin J. C. Barrett S. H. Yuspa	Staff Fellow Senior Staff Fellow Section Head LPFT NIEHS LPFT NIEHS EPB NCI
COOPERATING UNITS (if any) Experimental Pathology Branch, NCI		
LAB/BRANCH Laboratory of Pulmonary Function and Toxicology		
SECTION <u>In vitro</u> Carcinogenesis		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 1.25	PROFESSIONAL: 1.25	OTHER:
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (a1) MINORS ☐ (a2) INTERVIEWS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER		
SUMMARY OF WORK (200 words or less - underline keywords) The vast majority of human neoplasms is thought to be due to <u>environmental factors</u> . There is overwhelming evidence that <u>skin cancer</u> , one of the most commonly occurring human cancers, is usually due to exogeneous insult. The cells that are most likely to be progenitor cells for this carcinoma are the basal cells, which are rapidly dividing and/or differentiating, and as such make an excellent <u>target cell</u> for carcinogenic agents. The purpose of this work is (1) to develop an <u>in vitro</u> system consisting of dividing, basal cells isolated from epidermis, (2) to induce these cells to undergo neoplastic transformation <u>in vitro</u> following treatment with environmental carcinogens and/or promoters, (3) to isolate cells from skin papillomas and carcinomas induced <u>in vivo</u> , and (4) to compare the cellular properties of epidermal cells at various stages after <u>in vitro</u> treatment with carcinogens to cells derived from papillomas and carcinomas. The work will be done with inbred Syrian hamsters.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Epidermal cells are isolated from newborn Syrian hamsters (Telaco strain 87.20) by surgically removing the skin from the animals, floating the skin dermis side down on 0.25% trypsin for 24 hrs. at 4°C, physically peeling the dermis from the epidermis, and dissociating the epidermal cells by physical disruption. The epidermal cells are then grown in culture using a variety of culture conditions. Cells are exposed to carcinogens, promoters or both and then grown further and examined for various in vitro transformation phenotypes. In vivo experiments are performed by treating the skin of animals with carcinogens, promoters, or both. The animals are allowed to develop various neoplastic lesions. The activity of carcinogens and promoters is determined and compared with the in vitro results. Additionally, cell cultures are established from various neoplastic lesions such as papillomas and these cells are compared to cell cultures exposed to carcinogens and/or promoters in vitro.

MAJOR FINDINGS AND PROPOSED COURSE: Epidermal cell cultures from newborn Syrian hamsters have been established. The epithelial nature of these cultures was determined by morphological criteria but other epidermal cell characteristics are being examined. Various culture media and techniques are being compared to determine the optimum conditions for maintenance and growth of the cells.

Once proper culture conditions are established, the cultures will be treated with various carcinogens, such as benzo(a)pyrene, and the growth response and transformed character of these cells will be studied. Neoplastic and preneoplastic cell populations will be isolated and compared to the normal cells. Additionally, cell cultures will be established from neoplastic and preneoplastic lesions induced in vivo and compared to the in vitro treated cells. The progression of normal and premalignant cells to the malignant state will be studied and the effects of promoters, carcinogens, and mutagens on this process will be examined. The influence of fibroblasts on the development of neoplasia by the epithelial cells also will be examined.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Since skin cancer is one of the most prevalent human neoplasms, and one clearly influenced by environmental factors, finding a model to investigate the complex interrelationships between target cells, carcinogens, and promoting agents, as well as the progression from normal to preneoplastic to neoplastic cells would be of considerable value to the understanding of environmental carcinogenesis.

PROJECT DESCRIPTION

METHODS EMPLOYED: Oncogenesis of cells in culture is studied by examining the development of tumorigenicity and other in vitro properties of cells exposed to environmental agents. The model cellular systems under study include Syrian hamster embryo fibroblasts, mouse C3H 10T 1/2 fibroblasts, human skin fibroblasts (normal and transformed), and epithelial cells derived from trachea of the Syrian hamster and from tracheal carcinomas of this species.

MAJOR FINDINGS AND PROPOSED COURSE: Hamster embryo cells treated with environmental carcinogens in vitro undergo a multistep development of neoplastic transformation. Preneoplastic cell populations of such cultures have been isolated by selecting colonies with altered morphology, enhanced fibrinolytic activity or growth advantage under selective conditions. These preneoplastic cells are being studied in terms of their tumorigenicity, growth regulation in vitro, morphological features, proteolytic activity, karyotype, cAMP levels, and receptors for growth factors. The progression of these cells to the neoplastic state is being examined and the effect of known mutagenic insults on this transition is being studied.

The rates of spontaneous mutations of normal human fibroblasts and chemically transformed derivatives of these cells are being compared to determine whether transformed cells have higher spontaneous mutation rates.

The mechanism of action of 12-O-tetradecanoyl-phorbol-13-acetate (TPA), a potent tumor promoter, is being studied by determining whether TPA can mimic the action of growth factors on cells. Binding assays for several growth factors (epidermal growth factor, insulin, fibroblast growth factor, etc.) have been developed with cells which are known to respond to TPA. Whether TPA competes for these binding sites is under study. Additionally, the ability of these growth factors to act as promoters in the 10T 1/2 transformation system is being examined.

Benign and malignant tumor cells have been derived from hamster fibroblasts. The enzymatic activity of these cells is being studied and contrasted in hopes of determining the molecular basis for malignancy. Various in vitro assays of invasiveness are being developed. The systems currently being explored utilize isolated basement membrane from hamster lungs, the chick allantoic membrane or chick skin organ cultures. It is hoped that these assays will be able to differentiate between benign and malignant tumor cells. Studies on the mechanism of cellular invasiveness and on the progression of benign to malignant cells can then be conducted.

A normal epithelial cell line has been derived from hamster tracheal epithelium. The in vitro phenotypes of these cells are being studied and compared to cells derived from hamster tracheal carcinomas. The normal cells will be treated with carcinogens and promoters and followed for neoplastic development. Attempts to quantitate this process will be made by ascertaining the number

of cells capable of growing under various selective pressures.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Fifty to ninety percent of all human cancers are environmentally related. Before effective programs in cancer prevention and control can be formulated, a better understanding is required of the mechanisms by which environmental agents induce and promote neoplasia. Since cancer is primarily a cellular disease, this project utilizes model systems of cells in culture to study the cellular and molecular mechanisms of environmental carcinogenesis. Of particular interest is the cellular development of neoplasia. The progressive nature of neoplastic transformation recently has been recognized but the role of environmental agents in this process is unclear. This problem is central to environmental carcinogenesis and is the focus of this project.

PUBLICATIONS

Barrett, J. C. and Ts'o, P. O. P.: Mechanistic Studies of Neoplastic Transformation of Cells in Culture in Polycyclic Hydrocarbons and Cancer: Environment, Chemistry and Cell and Molecular Biology; ed. P. O. P. Ts'o and H. Gelboin, Academic Press, Inc. In press.

Barrett, J. C.: Progressive Nature of Neoplastic Transformation of Syrian Hamster Embryo Cells in Culture. Prog. Exp. Tumor Biol. In press.

PROJECT DESCRIPTION

METHODS EMPLOYED: In order to demonstrate malignant transformation, rat tracheal organ cultures were exposed on days 3 and 6 of culture to N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) for 6 hours. Epithelial outgrowths provided primary cell cultures which, after the appearance of morphologically altered cells were successfully subcultured into cell lines. The cell lines were then assayed for in vivo tumorigenicity by inoculating 10^6 cells into the thighs of immunosuppressed hosts. In a second experiment tracheal explants were first exposed to a concentration of MNNG which produces a very low frequency of transformation. The explants are then exposed to solvent only or various concentrations of 12-o-tetradecanoyl-phorbol-13-acetate (TPA) for 1 hour every 6 days for 3 weeks. Following these exposures, primary epithelial cell cultures and cell lines were established as before. At various times up to one year after exposure in vivo tumorigenicity was determined.

MAJOR FINDINGS AND PROPOSED COURSE: Our studies demonstrate, for the first time, that respiratory tract epithelium can be transformed in vitro by exposure to a chemical carcinogen, using an organ culture - cell culture system. The effect of the MNNG exposure alone was dose dependent with respect to the number of long term primary cell cultures obtained, the number of cell lines obtained, and the number of tumorigenic cell lines. No cell lines were derived from control cultures. After inoculation at approximately 10 passages, the average latency of tumor formation (or times between inoculation and tumor appearance) was dose dependent also (i.e., significantly longer in lower as compared to higher dose groups). With passage of the cell lines, we observe an increase in tumor incidence and a decrease in tumor latency. Keratinizing squamous cell carcinomas, adeno-squamous carcinomas, and undifferentiated carcinomas were obtained from cell lines in all groups.

In the second study we have observed the promoting effect of TPA in terms of increasing the frequency of tumorigenic cell lines and decreasing the time at which tumorigenicity could first be observed. Of the cell lines derived following exposure to MNNG alone, only 3 of 10 were tumorigenic between 300 days and one year. Cell lines derived from explants exposed to MNNG followed by multiple exposures to TPA were tumorigenic in 8 of 11 cases, three as early as 200 days.

Surprisingly, we were able to establish epithelial cell lines from explants exposed only to TPA. These cell lines are not tumorigenic when inoculated into immunosuppressed hosts. The cell lines will be exposed to a variety of carcinogens in vitro to assess their transforming capabilities.

In order to further quantitate our exposure system, we are using labeled TPA to measure distribution and metabolism in the organ culture system. We are also measuring the enhancement of DNA synthesis following TPA exposure using quantitative autoradiographic techniques.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In order to study the mechanisms of respiratory oncogenesis at the cellular level, in vitro systems such as the one described above will prove to be a valuable tool. Our in vitro model appears to be suited to test environmental agents for their oncogenic effects for respiratory tract epithelium and to study the interaction of agents and co-factors. We will also be able to search for morphological and biochemical markers to identify the various stages of carcinogenesis in respiratory tract epithelium.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25003-01 LPFT								
PERIOD COVERED										
TITLE OF PROJECT (80 characters or less) Tumor Induction Studies with the Tracheal Transplant Model										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT										
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI:</td> <td style="width: 33%;">D. Topping</td> <td style="width: 33%;">Staff Fellow</td> <td style="width: 33%;">LPFT NIEHS</td> </tr> <tr> <td>Other:</td> <td>P. Nettesheim</td> <td>Chief</td> <td>LPFT NIEHS</td> </tr> </table>			PI:	D. Topping	Staff Fellow	LPFT NIEHS	Other:	P. Nettesheim	Chief	LPFT NIEHS
PI:	D. Topping	Staff Fellow	LPFT NIEHS							
Other:	P. Nettesheim	Chief	LPFT NIEHS							
COOPERATING UNITS (if any) Pulmonary Carcinogenesis Program, Biology Division, Oak Ridge National Laboratory										
LAB/BRANCH Laboratory of Pulmonary Function and Toxicology										
SECTION Environmental Carcinogenesis										
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709										
TOTAL MANYEARS: 2.4	PROFESSIONAL: 1.3	OTHER: 1.0								
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS										
SUMMARY OF WORK (200 words or less - underline keywords) Our research efforts are directed toward investigating both the means of induction and the morphogenesis of cancer in the major airways of the respiratory tract. The model employed has been the heterotopic tracheal transplant which allows for quantitative exposure of circumscribed segments of rodent tracheal epithelium to chemicals and particulates of known or suspected carcinogenicity. Studies of changes in tracheal epithelium induced by known carcinogens suggest that cancers develop from certain focal lesions which arise long after cessation of carcinogen exposure. Studies with nickel and asbestos have demonstrated that both are irritant substances but have at most low carcinogenicity for tracheal epithelium. We are also in the process of (1) comparing the effect of the length of exposure to carcinogens vs. the total delivered dose through modification of the delivery system and (2) assessing the susceptibility of tracheal tissue from aged animals to chemical carcinogens. We are also assessing the applicability of the phenomena of promotion and co-carcinogenesis to the respiratory tract.										

PROJECT DESCRIPTION

METHODS EMPLOYED: Rodent tracheas are transplanted subcutaneously onto the backs of isogenic recipients. After the transplants establish, pellets containing test substances are inserted into the lumina of the tracheas. Chemicals are delivered to the trachea from pellets composed of beeswax matrix which contain the dissolved test chemicals. Pellets are either left in the tracheas or are removed at predetermined intervals to limit the period of exposure. Retrieved pellets are assayed to quantitate the chemical remaining in them. Rates of delivery of chemicals to tracheal transplants can be altered by either modifying the beeswax matrix or coating the pellets with polymer coatings. Particulate and insoluble materials are delivered by means of pellets in which gelatin is the supporting matrix.

MAJOR FINDINGS AND PROPOSED COURSE: The known carcinogens DMBA and BAP have previously been shown to produce tumors in tracheal transplants in a dose-dependent manner. We have determined that one milligram of benzo(a)pyrene is necessary to reproducibly induce squamous carcinomas in tracheas with a latent period of from 1 to 2 years. A dose of only 1/5th as much DMBA produces tumors with the same incidence. In the past year, we have been studying the lesions produced following 1 to 4 weeks of exposure to pellets containing 165 micrograms of DMBA. The lesions induced have been followed by means of a serial sacrifice study as well as a tumor induction study. Our findings suggest that reactions which occurred during the first 2 months after carcinogen exposure are primarily toxic in nature and that the pattern of progression of these changes are influenced as much or more by the length of exposure as by the total dose of the chemical delivered. Superimposed on these generalized changes were a number of focal lesions which appeared 3 to 4 months after initial exposure of the tracheas. The pattern of lesions which developed suggests that a number of them are involved in the evolution of cancer while others disappear without progressing to frank tumors. We are also using the tracheal transplant to investigate the carcinogenicity of nickel and asbestos for respiratory epithelium. For our studies we have used nickel subsulfide powder and UICC samples of crocidilite and chrysotile "A" asbestos. We have found 3 milligrams of nickel to be both toxic to the tracheal epithelium and sarcomatogenic, but have obtained only a low incidence of carcinomas in tracheas treated with 1 milligram of nickel.

Both types of asbestos have a pronounced irritant effect on tracheal epithelium and we have observed a high incidence of granuloma formation in asbestos treated transplants. Survival studies using transplanted tracheas treated with 2 milligrams of chrysotile "A" indicate that in this model asbestos may be sacromatogenic, but it has at most borderline carcinogenic activity.

We are also assessing the specific susceptibility of respiratory tissues from old animals to chemical carcinogens. To minimize the influence of the aged host, we have transplanted tracheas from 2 year-old rats into young recipients and have implanted them with DMBA-beeswax pellets. After one year, tumor incidence data are still fragmentary, but at the present time the incidence

appears similar in old and young transplants exposed to either 100 or 150 micrograms DMBA. During the next year, we will follow this tumor study to its conclusion. We are also planning ancillary studies to determine the capacity of cells from old tracheas vs. those from young animals to metabolize carcinogens and bind both the native chemicals and their metabolites to DNA.

Proven incidences of human cancer from a single agent are relatively rare, at least in relation to the total incidence seen in the general population. The hypothesis is widely accepted that combinations of factors to which we are exposed either concurrently or sequentially are of much greater importance in the development of cancer. We have, therefore, undertaken several experiments in which we are attempting to find whether the phenomena of co-carcinogenesis and promotion, which have been well demonstrated in mouse skin, are in fact applicable to respiratory epithelium.

To determine whether the initiation promotion sequence occurs in this system, we have chosen 2 chemicals which have known initiator and promotor properties, DMBA and TPA. Serial sacrifice data which we have collected have shown that there are far more advanced lesions in tracheas treated with both chemicals than in those treated with either agent alone. The tumor induction study is only underway since 8 months.

Benzo(e)pyrene, usually considered a noncarcinogen by itself, acts as a co-carcinogen when applied together with benzo(a)pyrene on mouse skin. The experiment we are conducting questions whether B(e)P exerts a similar effect on respiratory epithelium and more basically, whether the phenomenon of co-carcinogenesis can be demonstrated in tracheal epithelium. This experiment has progressed for over 18 months, and the results we have obtained indicate that B(e)P does not act as a co-carcinogen and may inhibit carcinogenesis. B(e)P by itself appears to be non-tumorigenic as well as nearly non-toxic to respiratory epithelium.

We also have a major two-stage carcinogenesis study underway which combines polycyclic hydrocarbon exposure sequentially with asbestos exposure. After 4 weeks of exposure to DMBA the tracheas were exposed to 0.2 milligram crysotile "A". To date this study has progressed 19 months. Carcinoma production is still low and no statement concerning the effect of asbestos can be made.

In the upcoming year we will continue to follow and evaluate the ongoing studies in the major categories described above.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Bronchogenic carcinoma is one of the leading causes of death in the developed countries of the world today. We are attempting in these studies to further our basic understanding of the development and causation of respiratory cancer. We have in the tracheal transplant model a system whereby epithelial tissues can be exposed for specified periods of time to a variety of agents singly, sequentially, or in combination. Since human exposure to atmospheric pollutants

is both extended in time and complex in its pattern, it will be extremely important to know whether the phenomena of promotability and co-carcinogenesis apply to the respiratory tract and whether time dose exposure relationships can be constructed using tracheal epithelium as a test system. The development and utilization of the tracheal transplant model will provide means by which a wide spectrum of environmental contaminants may be tested to determine in a quantitative manner their toxic and tumorigenic potential for the respiratory tract.

UNITED STATES ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION
OAK RIDGE OPERATIONS
Oak Ridge, Tennessee 37830

TITLE: Susceptibility of Preneoplastic Epithelial Lesions to Toxic and Carcinogenic Insults.

CONTRACTOR'S PROJECT DIRECTOR: A. J. P. Klein-Szanto, Ph.D.

PROJECT OFFICER (NIEHS): Paul Nettesheim, M.D., Chief, LPFT

DATE CONTRACT INITIATED: October 1, 1978

CURRENT ANNUAL LEVEL: \$165,000

PROJECT DESCRIPTION

OBJECTIVES: In order to approach with greater accuracy the problem of the susceptibility of preneoplastic epithelial lesions to toxic and carcinogenic insults, we are characterizing these lesions of the respiratory tract by using not only conventional morphological techniques, but also a combination of histochemical, electron-microscopical and morphometric methods. Each group of precursor lesions will be classified according to the histopathological features, at light and electron-microscopical levels, glycogen and mucosubstance content as detected by histochemical reactions, cell kinetics, and cell and nuclear morphometric size-indicators using computerized planimetric techniques. Although most of these studies are and will be carried out on one of the widely used experimental models developed in our laboratory, namely the tracheal transplant model, we are at the same time considering the use of other models which could be advantageous for a better categorization and staging of epithelial lesions of the respiratory airways, and at the same time constitute adequate models for susceptibility studies.

METHODS EMPLOYED: Heterotopically transplanted rat tracheas which were continuously exposed to measured amounts of DMBA for 1 to 6 months were embedded in Epon and processed for light microscopical (toluidene-blue) histochemical (PAS and Alcian blue) and electron-microscopical observations. Most of the transplant-bearing animals were also injected with ^3H -thymidine prior to sacrifice. A morphometric study of the 1-2 μm thick Epon sections of precursor lesions is being carried out using a planimetric graphics tablet connected to an on-line computer. A specially devised program permits the calculation of a series of cellular and nuclear size indicators. The parameters which are being analyzed are: mean cell surface, mean nuclear surface (indicators of cell and nuclear size respectively), and the nucleus: cytoplasm ratio.

MAJOR FINDINGS AND PROPOSED COURSE: Epithelial alterations induced by DMBA have been tentatively grouped in the following categories: 1) epithelial atrophy, 2) epithelial hyperplasia, 3) transitional metaplasia, 4) squamous metaplasia, 5) epithelial dysplasia, and 6) carcinoma in situ. This classification based on classical histopathological criteria will serve as a tool for studying these alterations by more elaborate techniques which will allow a better description of the changes taking place in the respiratory epithelium subjected to carcinogenic stimuli. Once the lesions are so characterized and the classification eventually modified, our studies will be directed to studying the influence of various agents on the evolution of preneoplastic lesions. In this context, we not only foresee the eventual utilization of in vivo models as the one used for our characterization studies, but also the use of combined in vitro - in vivo models which would permit a more synchronous production of in vivo epithelial lesions originated in cell clones previously characterized by in vitro techniques.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The possibility of obtaining a well-characterized and synchronous sequence of carcinoma-precursor lesions would be of great importance for further investigating the pathogenesis of respiratory tract neoplasms. In our case, the use of such a model would greatly facilitate the experimental design in order to determine if initiated epithelial cells show different susceptibility to carcinogenic or toxic insults during various stages of progression to neoplasia. The use of such a model would be of interest for studying the enhancement of neoplastic progression in carcinogen-exposed respiratory epithelium, by environmental toxins, relevant to the respiratory tract. The system should also lend itself to study methods of "intervention therapy" i.e., to develop approaches suited for inhibition of neoplastic progression.

RESEARCH RESOURCES PROGRAM

RESEARCH RESOURCES PROGRAM Summary Statement

The Research Resources Program provides the principal, technical, and scientific support capabilities to the Intramural Research Programs of the NIEHS. It provides scientific support in the areas of chemistry, comparative pathology, inhalation toxicology, laboratory animal medicine and husbandry, and research contracts coordination; engineering support through facilities planning, operation, and maintenance; as well as technical support through the operation of the NIEHS Library, NIEHS safety program, arts, graphics and photography, as well as glassware and media supply.

The Research Resources Program also plans, develops, and conducts research in targeted areas. This is accomplished independently or in collaboration with other NIEHS laboratories, Federal agencies, academic institutions, or contract laboratories.

Administrative Activities: A Comparative Medicine Branch was established. A search for a qualified and experienced veterinarian to direct this program resulted in the appointment of Dr. A. Edward as Branch Chief. Reorganization of the Environmental Biology and Chemistry Branch resulted in the establishment of Comparative Pathology, Inhalation Toxicology, and Chemistry Sections. The growth of the latter section is such that separate Branch status is planned. The hiring of an Administrative Officer within the Office of the Associate Director, RRP, was realized and will improve administrative practices within all operating segments. The developing Toxicology Bioassay Program realized significant advances through the successful recruitment of scientist vacancies. The planning and logistics for the beneficial occupancy of the permanent facilities due to be completed in 1980, was assigned to the RRP. Initial plans are underway to prioritize and initiate the ordering of essential equipment.

RESEARCH CONTRACTS

The NIEHS Research Contracts Program reflects an extension of Intramural Laboratory capabilities and interests. The Research Contracts Office coordinates the allocation of contract funds, peer review of contract experimental design, evaluation of contract proposals, and the monitoring of contracts upon award. A total of \$6.1 million was invested in over 40 research contracts in FY-78. One third of contract funds are allocated to three priority areas: the Mutagen Bioassay, Toxicology Bioassay, and Oral Asbestos programs. The quality and effectiveness of all research contracts is now considered by the NIEHS Board of Scientific Counselors who review research contract activities as part of their evaluation of intramural research activities.

NIEHS LIBRARY

The NIEHS Library is the principal science reference resource for the Institute. A professionally trained, experienced librarian was hired to plan and direct these activities late in FY-77.

Journals: The journal collection is the backbone of the Library. Scientists rely heavily on journals for information on current developments in their field of methodology. As a result of analyzing interlibrary loan requests, the Library subscribed to approximately 90 new serials in addition to about 325 already received. An inventory of the journal collection was made, and many missing issues were replaced through gifts and the Medical Library Association Exchange. The collection was reviewed and some inappropriate journals donated to other libraries.

The Library began a project with EPA to make a combined computerized journal holdings list. New catalogs of journal holdings can now be generated quickly.

The Library ordered the complete set of Chemical Abstracts (CA) on microfilm, freeing up about 36 shelves for other purposes. Also ordered was the complete set of Collective Indexes to CA, the older ones on microfilm. A current subscription to CA was also begun. This set is of major benefit to biologists as well as to chemists and librarians.

To conserve space, some back volumes of journals in microfilm were purchased. A new procedure for processing Branch subscription requisitions was established; the Library now coordinates these requests to avoid unnecessary duplication.

Books: In order to make all the Institute books accessible, the Library began an on-going project to catalog all books purchased by Laboratories/Branches. Library of Congress catalog cards are used for a subject/title file and an author file.

A faster and cheaper method for purchasing books was effected through establishing a Blanket Purchase Order with a medical book distributor. The distributor maintains a large inventory and provides a two week delivery at a 10% reduction of the publisher's price. The Library also eliminated back orders, some of which had been outstanding since FY-76, and initiated a claiming system for orders pending for more than three months.

The Library ordered more than 1200 books for the Institute in FY-78; approximately half were for the Library's collection.

Fedlink: The Library commenced developing an automated cataloging system through FEDLINK, a network of Federal Libraries coordinated by the Federal Library Committee at the Library of Congress. The FLC acts as an intermediary between Federal libraries and OCLC (the Ohio College Library Center) to provide computer access to the 5 million catalog

records maintained on the OCLC computer. The advantages gained from FEDLINK include: immediate access to catalog records from which catalog cards can be readily produced; one week turn-around time for delivery of these cards; production of a monthly computer tape of these cataloged items; and the possibility of a completely online card catalog. One non-cataloging advantage is that the online records indicate which libraries hold a particular book, useful for filling interlibrary loan requests.

Technical Reports: The Library began an automated cataloging of the technical report collection. This system generates printouts by author, title, originating agency, and subject. The Biometry Branch was instrumental in programming this project.

The Library was placed on mailing lists for NIOSH reports and for NCI bioassay reports. Past reports from these series were ordered on microfiche. Other reports are being ordered regularly from the National Technical Information Service and from the Government Printing Office.

Interlibrary Loan: In October 1977, the local university medical libraries substantially increased their charges for interlibrary loans and photocopies. A meeting of librarians from the Research Triangle health sciences libraries was held in November. At this meeting a compromise was reached. The smaller (NIEHS) libraries would send a staff member to do photocopying at UNC or Duke in place of sending requests for the academic libraries to fill. This relieved the workload for the academic libraries and is less expensive for NIEHS.

The Library expanded its capabilities by establishing accounts with the Institute for Scientific Information (publisher of "Current Contents") and the Linda Hall Science Library. We also acquired several basic union catalogs of journal holdings to expedite locating requested journals. One disappointment was the unwillingness on the part of the local academic libraries to allow us to establish a system so our individual scientists could visit those libraries and charge photocopies to an NIEHS account.

An important statistic was the 20% decrease from the first quarter to the second in the number of interlibrary loan/photocopy requests. The NIEHS now averages about 700 requests per month; this reduction is attributed to improvements in our collection and more careful attention to which libraries hold the journals or books.

An event much talked about in library circles was the advent of a new Copyright Law, January 1, 1978. This law imposed limitations on the quantities of articles that we could get through interlibrary loan channels. In actuality, these limits are not an imposition; however, the necessary paperwork for tracking purposes is considerable.

Reference/Literature Searching: Most research in the Library continues to be done by the bench scientists. However, the number of computerized literature searches performed increased by an estimated 30%. The number of regular monthly current awareness searches (SDI's) also increased,

reflecting active solicitation for this service on the part of the Library.

One particularly exhaustive search is noteworthy. A scientist requested toxicity and handling data on about 60 compounds being used in his lab. While we were able to find information on most of them, the search did underscore that there is not a single comprehensive source for toxicity information.

Another data base, Excerpta Medica, was added to the approximately 90 other data bases accessible by the Library. It promises to be an excellent complement to Medline (Index Medicus) and Biosis (Biological Abstracts).

Miscellany: A monthly newsletter and acquisitions list was begun in January. Each month it focused on one specific aspect of the information services available to NIEHS personnel. The acquisitions list immediately caused an increase in circulation.

A new brochure describing Library services was designed and distributed. The Librarian met with most of the Branches to discuss these services and solicit suggestions.

Although some rearranging of shelves was accomplished, the lack of space is the major problem faced by the Library. A second Xerox machine was ordered to relieve photocopying pressures, since the single machine averaged more than 30,000 copies per month--almost twice the recommended number.

Summary: Much attention this year was given to planning and upgrading the collection and services. This involved a large increase in the budget, some of which was for one time purchases and temporary staff. To maintain the level-of-effort and the quality of the collection in future years will require expenditures not appreciably less than that for FY-78. As improvements are made in the quality of a service organization, demand increases proportionately, and the Library is in a position which not only requires a high quality of service but also requires planning for the upcoming expansion of the Institute. FY-78 was a year of building, of filling in gaps, of trying new methods, and of laying the groundwork for further building over the next several years.

OFFICE OF SAFETY

The Institute commitment toward an effective Safety Program realized tangible gains in a number of areas. The availability of three qualified, experienced safety officers accounted for some major advances. However, the active participation of numerous NIEHS employees is a most significant factor in bringing the variety of efforts to fruition at an accelerated rate. Major advances are:

Physical Plant: A high hazard containment facility was completed in Building 14. A high hazard chemical synthesis laboratory was completed in Building 2. The west and north sectors of Building 7 are being

renovated to provide space that is adequate for complete laboratory operations. Potentially hazardous laboratory activities were relocated from Building 7 to laboratories better adapted for work of this sort. An enclosed covered area was constructed for the packing and storage of radioactive and chemical wastes. The building can be locked to prevent unauthorized access.

Environmental Monitoring: A limited monitoring program for potential workplace hazards is currently underway. It involves the use of colorimetric indicator tubes for specific contaminants and sound level measurements for occupational noise.

Safety Committee: A Safety Committee was formally chartered. It has actively participated in developing and reviewing safety policy. It reviewed the laboratory operations of Building 7 to insure their compatibility with the limitations of the building's current mechanical system.

Safety Manual: A comprehensive safety manual was developed and is to be issued to each NIEHS employee. The manual, specific for the chemical agents and research procedures of NIEHS, was prepared by the NIEHS Safety Committee and Safety Officers.

General Safety: Formal inspection of all facilities were initiated by the Safety Officer. Reinspections are conducted quarterly. Written reports are furnished so that all concerned will be aware of existing conditions that are to be corrected.

A general laboratory safety course, designed primarily for laboratory personnel, is being taught by the Safety Officer on a quarterly basis. Typical subjects covered include: OSHA and Federal employees, routes of entry of toxic agents, personal protective devices, proper use of laboratory hoods, safe handling of dangerous chemicals, special or unusual laboratory hazards, and safe use of compressed gas cylinders.

Radiation Safety: Four eight-hour introductory radiation courses have been held with a total of more than 50 people attending. Several investigators have taken intensive one-week training at NCSU. Surveys have been conducted monthly of all laboratories using radioisotopes. Over 50 principal investigators now use radioisotopes in their studies.

The Institute applied for and was granted an NRC license of broad scope to replace our previous highly limited license. The Radiation Safety Committee continues to review applications for the use of radioisotopes and actively investigates all accidents.

All shipments of radioisotopes are received by the Radiation Safety Office and swipe tested for contamination and leakage. About 300 shipments of radioisotopes were processed last year. Waste pickup procedures have recently been modified to provide for quicker disposal of accumulated wastes.

COMPARATIVE MEDICINE BRANCH

COMPARATIVE MEDICINE BRANCH Summary Statement

The Comparative Medicine Branch was established and Dr. A. E. Edward commenced duties as Branch Chief on July 1, 1978. Dr. Edward was Professor and Chairman of the Dept. of Comparative Medicine, School of Medicine, Wayne State University, Detroit, Michigan, prior to joining the Institute.

The mission of the Comparative Medicine Branch is to program and coordinate experimental animal procurement, housing, and utilization within the Institute; develop, refine, and advise Institute scientists and programs of appropriate animal models for use in Institute research programs; maintain collaborative laboratories in microbiology, experimental surgery, laboratory animal medicine, and mammalian reproduction; and plan and conduct research appropriate to these laboratory functions.

Administration: The CMB continued to review and develop plans and specifications for the permanent site facility currently under construction. The facilities will add 81,860 gross square feet of research animal space to existing facilities of 24,353 square feet.

Plans were submitted for the construction of a rodent isolation facility of 2,880 square feet. Equipment for the facility has been ordered. Resident breeding colonies from Building 15 will be transferred to this facility to better preserve their specific pathogen free status. The Building 15 space currently occupied by these stocks will be recommitted to the housing of animals used in direct research.

The paucity of animal space at NIEHS resulted in the Institute contracting with Duke University for the housing of its rabbits and larger animal species such as primates, horses, ponies, sheep, and goats. Limited quantities of rats and mice are also housed at Duke University.

Efforts to involve animal care personnel in the overall participation of projects have developed well. Animal care employees are more actively involved in assisting scientific staff in the technical aspects of their studies, which have improved technical skills and interest. Participation in the AALAS training course has resulted in the certification of four employees at the Animal Technician level and two at the Assistant Technician level. Currently, four employees are attending classes.

The continued availability of healthy rat and mice genotypes is still a principal concern. The NIEHS has established a policy of using inbred rats and mice of the NIH genotype. A research contract is being awarded that will derive pedigreed animals (from strains selected and provided by NIEHS) in a disease free state using gnotobiotic techniques. These animals will be maintained in isolators until shipment to NIEHS. In selected instances, strains will be expanded in pathogen free rooms for the production and supply of expanded numbers of animals. The objective is to provide animals of a quality and quantity that is required by

NIEHS research. The use of animals of NIH genotype allows one to capitalize on the genetic repository maintained by DRS, NIH, and insures subline compatibility with a number of other programs at NCI where we are developing collaborative research.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30008-07 CMB									
PERIOD COVERED October 1, 1977 to September 30, 1978											
TITLE OF PROJECT (80 characters or less) The Effects of Environmental Chemicals on Host Resistance to Infectious Agents											
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 60%;">J.E. Thigpen Microbiologist</td> <td style="width: 25%;">CMB NIEHS</td> </tr> <tr> <td>OTHER:</td> <td>E.E. McConnell Veterinary Pathologist</td> <td>EBCB NIEHS</td> </tr> <tr> <td></td> <td>J.A. Moore Supervisory Veterinary Medical Officer</td> <td>EBCB NIEHS</td> </tr> </table>			PI:	J.E. Thigpen Microbiologist	CMB NIEHS	OTHER:	E.E. McConnell Veterinary Pathologist	EBCB NIEHS		J.A. Moore Supervisory Veterinary Medical Officer	EBCB NIEHS
PI:	J.E. Thigpen Microbiologist	CMB NIEHS									
OTHER:	E.E. McConnell Veterinary Pathologist	EBCB NIEHS									
	J.A. Moore Supervisory Veterinary Medical Officer	EBCB NIEHS									
COOPERATING UNITS (if any) Animal Husbandry Section, CMB Joe Haseman, Ph.D., EBB Histology Section, EBCB											
LAB/BRANCH Environmental Biology and Chemistry Branch											
SECTION Comparative Medicine Branch											
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709											
TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.6	OTHER: 0.4									
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS											
SUMMARY OF WORK (200 words or less - underline keywords) Previous studies have shown that mice exposed to TCDD (5ug/kg body weight) once weekly for 4 weeks, are more susceptible to infection, and that 5-day Thioglycollate induced peritoneal exudates contain significantly less lymphocytes and macrophages than controls. Present investigations are geared to determine whether this reduction in peritoneal cells is due to impaired chemotactic function or due to an antiproliferative effect on pro monocytes or macrophages. Either effect could contribute to increased susceptibility to infection with <u>Salmonella bern.</u>											

PROJECT DESCRIPTION

METHODS EMPLOYED: (Blind Study) Four-week old male C57BL/6JFH mice will be randomly divided into 2 groups. Each mouse will be weighed and dosed via gavage once each week for 4 weeks with TCDD in acetone-corn oil or with only acetone-corn oil. Two days after the last dose of TCDD, a leukocytic exudate will be induced in the peritoneal cavity with 2.0 of thioglycollate. Mice will be sacrificed at 0, 3, 6, 12, 24, 48 and 120 hours and heart blood, peritoneal exudates, and bone marrow cells collected for total and differential cell counts. Macrophages will be tested for chemotactic function.

MAJOR FINDINGS AND PROPOSED COURSE: Preliminary studies for measuring spontaneous and directional migration (chemotaxis) of macrophages have been completed. We are ready to begin with TCDD treated animals. These studies will continue.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A variety of environmental chemicals when administered in sublethal doses are immunosuppressive and increased mortality rates have been observed upon exposure to infectious agents. These findings are significant in that humans may be exposed to these same compounds in minute quantities and, therefore, may be rendered more susceptible to certain types of infectious agents. It is important that we establish why mice exposed to TCDD are more susceptible to bacterial infections.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30078-01 CMB																
PERIOD COVERED July 1, 1977 to September 30, 1978																		
TITLE OF PROJECT (80 characters or less) Health Surveillance of Laboratory Animals at NIEHS																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																		
<table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 30%;">D. B. Feldman</td> <td style="width: 40%;">Veterinary Medical Officer</td> <td style="width: 20%;">CMB NIEHS</td> </tr> <tr> <td>OTHER:</td> <td>B.N. Gupta</td> <td>Veterinary Medical Officer</td> <td>EBCB NIEHS</td> </tr> <tr> <td></td> <td>J. E. Thigpen</td> <td>Microbiologist</td> <td>CMB NIEHS</td> </tr> <tr> <td></td> <td>G.A. Boorman</td> <td>Veterinary Medical Officer</td> <td>EBCB NIEHS</td> </tr> </table>			PI:	D. B. Feldman	Veterinary Medical Officer	CMB NIEHS	OTHER:	B.N. Gupta	Veterinary Medical Officer	EBCB NIEHS		J. E. Thigpen	Microbiologist	CMB NIEHS		G.A. Boorman	Veterinary Medical Officer	EBCB NIEHS
PI:	D. B. Feldman	Veterinary Medical Officer	CMB NIEHS															
OTHER:	B.N. Gupta	Veterinary Medical Officer	EBCB NIEHS															
	J. E. Thigpen	Microbiologist	CMB NIEHS															
	G.A. Boorman	Veterinary Medical Officer	EBCB NIEHS															
COOPERATING UNITS (if any) Animal Husbandry Section, EBCB																		
LAB/BRANCH Comparative Medicine Branch																		
SECTION Comparative Biology Section																		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																		
TOTAL MANYEARS: 3.5	PROFESSIONAL: 1.2	OTHER: 2.3																
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																		
SUMMARY OF WORK (200 words or less - underline keywords)																		
Incoming and resident animals at NIEHS are monitored for morphological abnormalities, <u>spontaneous diseases</u> and <u>parasitism</u>. The health status of research animals is vital to reliable interpretation of experimental data. Sporadic respiratory disease among <u>laboratory rodents</u> is associated with Sendai virus, usually in association with <u>Pasteurella pneumotropica</u>. Enteric pathogens have not been detected. Pinworm infections are infrequent. No pathologic lesions can be attributed to these nematodes. Pasteurellosis in NZW rabbits has not been encountered since purchasing them from a new source. Infectious disease in <u>guinea pigs</u> or <u>hamsters</u> has not been encountered.																		

PROJECT DESCRIPTION

METHODS EMPLOYED: All species and strains of research animals at NIEHS are routinely observed for clinical signs of disease.

New species or strains of animals from new sources undergo complete monitoring, including examination for respiratory and enteric pathogens, ecto- and endoparasites and incidental pathology. Reference histological slides are kept on each strain or species. Pooled sera are periodically screened for viral serology.

Rodents in breeding colonies are surveyed monthly, other resident animals are monitored approximately every 8 weeks for respiratory pathogens and parasites. Tissues from major organs are collected for histopathologic examination. Fecal samples are collected weekly from individual shipping crates and examined by the flotation method for parasite eggs. Positive results confirm infestation at the supplier.

Dead, diseased or abnormal animals are usually submitted to necropsy, after clinical examination.

MAJOR FINDINGS AND PROPOSED COURSE: Incoming and resident rats monitored at monthly intervals have been free of salmonellosis and chronic respiratory disease (CRD). Occasionally, newly received weanling rats revealed focal corneal opacity and periorbital edema. This condition has proved to be resolving stages of sialodacryoadenitis, a transient viral infection. Lesions usually disappear completely within a week.

Swiss and inbred mice have been relatively free of respiratory infections. Variable titers to Sendai virus are encountered but lesions attributable to this respiratory virus are uncommon. Pinworm infections are sporadically encountered but clinical cases of diarrhea or prolapsed rectum have not been seen. The presence of this parasite is esthetically displeasing but eliminating the infection is extremely difficult to accomplish. Hamsters have been relatively disease free. Guinea pigs purchased from a reliable SPF colony have been free of infectious disease. The incidence of "snuffles" (pasteurellosis) in NZW rabbits has virtually been eliminated since changing source of supply. During the period of 12 months (April, 1977 to March, 1978) 1,162 animals were necropsied for health surveillance. Surveillance of laboratory animals will continue on a routine basis.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

The health status of all research animals must be continually monitored; otherwise, intercurrent infections could cause misinterpretation and possible negation of valuable experimental data. In addition, microbiological and histopathological surveillance has uncovered interesting and valuable data heretofore unpublished in laboratory animal medicine.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30080-01 CMB
PERIOD COVERED July 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Simplified Gastric Intubation in the Rabbit		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: D.B. Feldman Veterinary Medical Officer CMB NIEHS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Comparative Medicine Branch		
SECTION Comparative Biology Section		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS:	PROFESSIONAL: 0.5	OTHER:
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) <u>Gastric intubation</u> is frequently employed in laboratory animals as a method of administering precise doses of liquids. This technique is difficult in the rabbit because of limited oral space. A technique was developed to simplify this procedure by utilizing a 13 gauge curved stainless steel feeding needle which is bent to approximate the curvature of the rabbit throat. By using a rigid tube that has been properly shaped, the likelihood of entering the trachea is minimized.		

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30081-01 CMB
PERIOD COVERED October 1, 1977 to 30 September 1978		
TITLE OF PROJECT (80 characters or less) The Effect of Dietary Protein on Lifespan, Frequency of Nephritis and Degree of Hypertension in the Golden Syrian Hamster		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: D.B. Feldman OTHER: E.E. McConnell J. Knapka G.A. Boorman	Veterinary Medical Officer Veterinary Pathologist Nutritionist Veterinary Medical Officer	CMB NIEHS EBCB NIEHS VRB NIH EBCB NIEHS
COOPERATING UNITS (if any) Animal Husbandry Section		
LAB/BRANCH Comparative Medicine Branch		
SECTION Comparative Biology Section		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.25	PROFESSIONAL: 0.2	OTHER: 0.05
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this study is to determine the effect of dietary <u>protein</u> <u>levels on lifespan</u> of the hamster as related to the severity of <u>nephritis</u> . An attempt will also be made to correlate the degree of nephritis with hypertension. Weanling male and female golden Syrian hamsters will be fed either 6, 12, 18 or 24 percent protein in a pelleted ration. The mean lifespan of each group will be calculated and survivors will be killed when 20% of a group remains. All hamsters will be necropsied and the kidneys will be examined histologically for nephritis. Other major organs will also be examined for lesions. Prior to necropsy of survivors, blood pressure will be determined by cannulation of the carotid artery.		

PROJECT DESCRIPTION

METHODS EMPLOYED: 180 (90 male and 90 female) weanling golden Syrian hamsters will be divided into 4 groups, each group receiving either 6, 12, 18 or 24 percent dietary protein ad libitum in a pelleted ration. The feed will be formulated every 90 days and analyzed for protein, fat, carbohydrates, lead, arsenic, cadmium, mercury, aflatoxins and antibiotics.

The animals will be housed individually in plastic cages (8' x 5' x 5' high) with hardwood bedding. Water will be supplied ad libitum in plastic bottles with stainless steel sipper tubes. Feed will be supplied in overhead cage top feeders. Weight gains will be recorded weekly on each animal until body weight plateaus, or does not increase more than 1% of the total body weight. Room temperature will be controlled ($70^{\circ}\text{F} \pm 2^{\circ}$ plus R.H. 50%) and lighting will be on a 12 hour light-dark cycle.

Complete necropsies will be performed on all dead or moribund animals, and histopathology will be done on a complete set of tissues. Major organs will be weighed. Blood will be collected at necropsy for hematology and serum chemistry. Urine will also be collected at necropsy and examined with standard multi-dip stick tests. Specific gravity and microscopic examination of sediment will also be performed.

The duration of survival of each group will be recorded and the differences compared by statistical methods. When 20% of a group remains, the survivors will be anesthetized with ketamine hydrochloride and halothane and blood pressure will be recorded after cannulating the carotid artery. This will be followed by complete necropsies, as above.

MAJOR FINDINGS AND PROPOSED COURSE: This project is due to commence shortly. The results of this project are to be published.

SIGNIFICANCE TO BIOMEDICAL RESEARCH OF THE PROGRAM OF THE INSTITUTE: NIEHS is increasing chronic toxicologic studies. It is imperative that research animals on these studies reach their maximum lifespan. Therefore, factors that increase or decrease mean lifespan in research animals is essential data. Hamsters are traditionally fed diets formulated for rats or mice, and which contain approximately 20% protein. Reducing the level of protein will delay the onset of nephritis in aging rats, but this has not been determined in the hamster. Hamsters on a lifetime carcinogenesis study die at a mean age of 56 weeks. Fifty percent die between 42-64 weeks, and fewer than 20% survive to 18 months. The reported lifespan of the hamster is 606-730 days. These animals are fed NIH-31, a diet containing 18-20 percent protein. This level may be too high for lifetime studies in the hamster.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30082-01 CMB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Comparison of Hamster Growth Associated with Hoarding and the Inability to Hoard Feed		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: D.B. Feldman Veterinary Medical Officer CMB NIEHS		
COOPERATING UNITS (if any) Animal Husbandry Section		
LAB/BRANCH Comparative Medicine Branch		
SECTION Comparative Biology Section		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.2	OTHER:
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) This project is to compare <u>feed consumption</u> and <u>weight gain</u> in <u>golden Syrian hamsters</u> fed through <u>overhead cage feeders</u> versus <u>feed placed directly into the cage (ability to hoard)</u> . The study will continue for 6 months, after which mean body weights of experimental groups will be compared. A difference of 20% in body weight will be considered statistically significant.		

PROJECT DESCRIPTION

METHODS EMPLOYED: The animals will be divided into 2 groups. Group 1 will receive feed ad limitum through the feeder cage top; group 2 will have pelleted feed put directly onto the cage floor. Water will be supplied ad libitum. Feed consumption and animal body weights will be calculated weekly. At the end of 6 months, both groups will be compared for differences in mean body weight. A difference of 20 grams will be considered statistically significant (20% of body weight). The results of this study will be published.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Hamsters are being used at NIEHS in increasing numbers for chronic studies. It is important that these animals reach both their maximum growth and life span. If hamsters thrive better having the ability to hoard feed, this method of feeding will be employed, rather than using cage feeders.

ENVIRONMENTAL BIOLOGY AND CHEMISTRY BRANCH

ENVIRONMENTAL BIOLOGY AND CHEMISTRY BRANCH

Summary Statement

The Environmental Biology and Chemistry Branch maintains collaborative support laboratories in chemistry, comparative pathology, and inhalation toxicology. It also provides the principal laboratory capabilities of the NIEHS Toxicology Bioassay Program. The Branch also plans and conducts research appropriate to laboratory capabilities.

Administratively, the Branch is currently organized into three sections: Chemistry, Comparative Pathology, and Inhalation Toxicology. The Animal Husbandry Section, Microbiology Laboratory, and veterinary medicine capabilities were transferred from EBCB to the Comparative Medicine Branch. The Environmental Teratology Information Center (ETIC) and the contract teratology screening activities were reassigned to the EBCB in the second half of the year. These latter activities will be incorporated into the developing toxicology bioassay capabilities.

TOXICOLOGY BIOASSAY

The NIEHS oral asbestos studies on the biologic effects of ingestion of selected forms of asbestos are being conducted using rats and hamsters. These studies are performed under contract. The final reports of extensive chemical and morphologic characterization of the selected asbestos forms are to be received late in the year. The NIEHS has provided numerous asbestos samples to other governmental and academic organizations for use in their research programs.

A collaborative study on the effects of inhaled asbestos was begun with the Pneumoconiosis Research Unit, Medical Research Council, United Kingdom. Using an agreed upon protocol, UICC Chrysotile B and fibrous glass are being studied in rats. NIEHS Short Range Chrysotile and NIEHS Intermediate Range Chrysotile are also being studied using the same exposure conditions. The inhalation phase of the study is being performed under contract and the pathologic interpretation will be performed by NIEHS.

A major focus of activity has been interaction with the NCI Carcinogen Bioassay Program. Meeting with the NCI Experimental Design group on a monthly basis, NIEHS scientists review and evaluate the design of proposed carcinogen studies. In selected instances, protocol modifications have been effected that will evaluate the toxicologic properties of chemicals under test.

A research protocol has been developed to study the increased sensitivity of laboratory animals to carcinogenic and toxicologic effects of chemicals when exposed at various dose levels during in utero development and their postnatal life. These data will be compared to data simultaneously collected from animals exposed only during the post weaning phase of life. Three or four chemicals will be evaluated through contract research.

NIEHS is conducting a series of experiments to assess the spectrum of toxicologic effects associated with PBB. Reports describing analytical chemistry, toxicology, distribution and excretion, immunology, and

behavioral effects are to be found in individual project narratives. Experiments to determine the effects of activated charcoal, cholestyramine, and caloric deprivation on PBB body burden are scheduled for the last quarter of the fiscal year.

CHEMISTRY

Environmental Chemistry deals with the behavior of environmental agents and their effects on the physical environment and the biosphere. At NIEHS, the Chemistry Section focuses on delineating the chemistry of environmental agents which show undesirable biological effects on mammalian systems which serve as models for man. Emphasis is placed on manmade (xenobiotic), persistent molecules with particular attention on the halogenated organics and the lipophilic xenobiotics.

Support Activities

Environmental chemistry interfaces environmental analysis, detection and measurement of environmental agents, with environmental toxicology, detection and measurement of various deleterious biological activities of environmental agents. Therefore, chemists are frequently consulted within NIEHS to advise biological scientists in appropriate areas of their research. The support activities of the Chemistry Section, as expected, fluctuate depending upon the resources required to meet a particular need. Chemistry support requests are increasing in terms of the number and the degree of involvement. The requests generally fall into one of two categories: short term requests such as compound purity determinations and organic synthesis by published procedures, and a variety of long term requests which often require new and improved methodology development such as qualitative and quantitative analysis of complex mixtures and organic synthesis by unpublished procedures. In the latter case it is difficult to determine the amount of time and effort which will be necessary to complete the task. A trial period may be necessary to demonstrate feasibility within the limitations of the group. Therefore, for obvious reasons these latter requests are carefully evaluated, and we frequently seek to do this work on a collaborative research support basis.

Chemistry support generally involves the use of established known methodologies as opposed to the development of new ones. It will consider developing new methods when it appears to be particularly relevant to ongoing programs and is appropriate to the expertise of the group. Special consideration is given to requests that cannot be done outside NIEHS or where clearly we can do it better. In addition, Chemistry provides support in securing procurement and research contracts of a chemical nature. Chemistry support requests are assigned to one of three work groups. These work groups also form the basis for research activities within the Section; viz., Analytical Biochemistry, Bioorganic Chemistry and Synthesis, and Specialty Instrumentation.

The largest number of analytical determinations (over 2300) was made by the Specialty Instrumentation group using gas chromatography-mass spectrometry (GC/MS). About 39% of these requests were for routine

service, with the remainder in collaborative support of various biological studies, instrumental or method development research. Only a small percent of the total number of samples run were performed using the high resolution mass spectrometer and data system. This number is expected to increase as this relatively new system continues to undergo stabilization.

Procedures have been established to facilitate the use of Chemistry Section high pressure liquid chromatography (HPLC) equipment by other Institute staff. Over 230 analyses have been performed through scheduled use of this equipment.

The Bioorganic Chemistry and Synthesis group handled a number of different samples including NMR analysis of a variety of chemical and biological metabolites and in many cases observed both ^1H and ^{13}C nuclei using ^1H decoupling and fourier transform (FT) techniques. Most of these samples were generated from other projects within the section. The use of the NMR facility by Institute staff outside of Chemistry is expected to increase with the addition of research programs requiring a greater degree of mechanistic study at the molecular level.

The more difficult or involved synthetic and/or preparative requests included: aborted attempts to prepare the catechol derivative of diethylstilbestrol, synthesis of ^{14}C -labeled 1,2-methylene (^{14}C) dioxy-4-propylbenzene, selected brominated biphenyl and unsymmetrical chlorinated biphenyl isomers, and O-carboxymethyl estrone.

A number of other requests were made for services of the Analytical Biochemistry group which required specific methods. These ranged from specific analyses for DNA, lipid classes and fatty acid compositions, specific isomer content, metabolites and purity and radiopurity of unlabeled and labeled research chemicals to syntheses, biosyntheses and determination of reaction rates and reactivity profiles. These types of analyses totaled over 800 samples of which the majority were for DNA analysis.

Chemical management services facilitated the procurement, storage, dispersal, and where necessary, disposal of specialty research chemicals, particularly with respect to mutagenicity testing programs.

Research Activities

The nature of the research in the Chemistry Section is to some degree, predetermined by its support commitment and space limitations. For example, the Chemistry Section attempts to provide broad areas of science competence rather than indepth and narrow science expertise. However, this requires the availability of diverse instrumentation which consumes space and requires routine maintenance. There are five major areas of research involvement in the Chemistry Section which are using selected classes of chemical compounds as models and/or special chemical, biochemical and instrumental techniques to develop general methodology of value to environmental health scientists.

Immunochemistry and Immune Functions: Immunoassay methods are being developed which will offer extreme specificity and sensitivity to the detection of hazardous environmental agents and their residues as well as their biological insults such as mutated proteins, etc. at biologically meaningful levels. Radioimmunoassays (RIA) have already been developed for chlorinated dibenzodioxins and mono-2-ethylhexyl phthalate. Progress has been made in developing RIA's for specific chlorinated biphenyl and dibenzofuran isomers. Related studies have indicated that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) directly effects lymphocytic function in mouse spleens. This approach offers promise as a new bioassay procedure for the dioxins and related compounds as well as the direct study of effects on cell function including possibly the primary action of TCDD on the cell. Other studies have examined the effects of selected environmental agents on the immune system in various animal species. The chemicals studied include polybrominated biphenyls (PBB's - immunosuppression in mice and rats), 2,3,7,8-tetrachlorodibenzofuran (TCDF - immunosuppression in guinea pigs), pure and technical grades of pentachlorophenol (PCP - studies in progress in cattle), diethylstilbestrol (DES - immunosuppression in mice exposed in utero), and lead (Pb - immunosuppression in rats).

Mechanism Elucidation: There is increasing evidence that certain highly toxic halogenated hydrocarbons may have specific binding receptors in biological systems which differ quantitatively in their ability to bind both halogenated and non-halogenated planar molecules. Binding propensity and toxicity may be correlatable. Chemical and biochemical studies are being conducted to provide an understanding of the specific molecular level interactions involved in binding which may in turn permit one to predict, prevent, or reverse them. Exact molecular structures have been determined by X-ray crystallographic measurements or estimated based on postulated structures. These data suggest the importance of molecular symmetry and size as determined by the number, kind, and positions of halogens; planarity; interatomic distances and overall stereoelectronics in biological activity. The hexachlorobiphenyls as model PCB's continue to undergo study. Recent work has shown different structure-dependent effects on lipid metabolism and cell structure as well as on induction of various enzyme activities in rat liver. These studies are supported by synthetic methods development to afford a variety of halogenated aromatic compounds as models including the assumed reactive intermediary metabolite arene oxides and related oxygenated derivatives. Regio-specificity and stereospecificity studies of the chemical conjugation of benzo(a)pyrene oxides (some specifically labeled with ^{14}C) to glutathione and related systems have also been initiated. Other work includes evaluating the capabilities of various tissue lipases and esterases as early mediators in the interactions of animals with xenobiotics containing ester linkages. These enzymes are also used as models in studies of protein-ligand interactions. Recent studies have led to partial characterization of the enzyme in human plasma responsible for the hydrolyses of phthalate ester plasticizers and will facilitate the pharmacokinetic elaboration of such chemicals in man. In the area of inorganic environmental agents, early work using nuclear magnetic resonance (NMR) spectroscopy has indicated that ^{207}Pb NMR may be a very useful probe into the specific binding of lead.

Mass Spectral (MS) Methods: Mass spectrometry (in combination with gas chromatography) is still the most sensitive, specific and versatile analytical method available for dealing with complex samples of environmental/biological origins. Negative chemical ionization MS techniques are continuing to be developed for the more sensitive and specific analysis of environmental agents. Studies of organophosphorus pesticides, nitriles and polychlorinated anisoles as models for the polychlorinated dioxins and diphenyl ethers have been completed and in general offer improved methodology for their analysis. Field-free region studies with selected environmental agents using high resolution mass spectrometry with metastable scanning has shown considerable promise in providing specific structural information useful in residue analysis as well as in establishing "chemical appearances" of environmental agents. Selected adducts of small polynucleotides and carcinogens/mutagens have been obtained for study by field desorption mass spectral techniques. Such studies should illuminate the micro-environments important in environmental chemical carcinogenesis. Mass spectral interpretations are being aided by molecular orbital calculations to determine minimum energy configurations of neutral molecules and positive and negative ions.

Other Analytical Methods Development: It is often necessary to develop and refine methodology for quantitative and qualitative determination of compounds and classes of compounds of general interest to the Institute and specific interest to individual investigators. Work in this area has emphasized development and validation of methods for quantitative and reproducible extraction and clean-up of halogenated aromatic compounds from biological materials for subsequent GC-MS or immunoassay, and analysis of spontaneous breakdown products of unstable xenobiotics. The former methods were useful in the analysis of rice oil contaminated with PCB-based heat exchange fluid in Japan (Yusho oil) and preliminary analysis of new and used PCB-based transformer fluids from this country.

¹³C-Nuclear magnetic resonance (NMR) was used to characterize several chlorinated polycyclodiene pesticides and some of their phototransformation and potential metabolic products and was shown to be superior to ¹H NMR for identification of these compounds and elucidation of their peculiar electronic interaction which underlie their biological activity. ¹H- and ¹³C-NMR were also used to characterize toxic fungal metabolites isolated from cereal crops.

Synthetic Methods Development: Expertise in synthetic organic chemistry is highly desirable for supporting a variety of research programs at the Institute. Not only are a diverse nature of custom organic chemicals required but they must also be of high purity. Such syntheses may also require stereospecific techniques since optically pure forms may be desired for study. In this regard, a chiral chemical synthesis of thromboxane B₂ (active intermediates in prostaglandin metabolism) has been completed. In the course of this work some specific derivatization methods for primary or secondary alcohols which permit selective transformations of these functionalities in the same molecule were developed. These latter methods are of general synthetic utility.

Other Activities

The Chemistry Section continues to provide unique analytical chemistry support to the study of environmental contamination problems of imminent public health concern. For example, the Institute has embarked on an epidemiological study to evaluate the health effects on infants of specific components of infant foodstuffs, particularly chlorinated pesticide residues and polychlorinated biphenyls (PCBs) in breast milk. This study requires well designed sample collection, analysis and data interpretation schemes. In this regard, the Chemistry Section has reviewed and evaluated current methodologies for measuring total organic chlorine and PCB residues in body fluids and tissues. Specific methods have been identified, improved, and recommended for use by contractors in providing analytical chemistry support to this large study. A significant amount of time has also been devoted to developing procedures for the contaminant-free collection, storage and dispensal of these human samples in order to insure long-term credibility of the analytical results. Significant progress has also been made toward development of an analytical methods quality control and assurance system including incorporation of new and improved methodologies consistent with providing the most suitable and reliable analytical data for answering the epidemiological question.

Other contract work is attempting to provide ^{14}C -labeled 2,3,7,8-tetrachlorodibenzofuran for biological study with respect to species differences in adsorption, metabolism, distribution and excretion. The furans are associated highly toxic impurities of the commercial PCB preparations.

The Section Office continues to evaluate the cost versus procedure benefit relationships for various custom synthesized chemicals (labeled and unlabeled) obtained on contracts. Necessary water purification equipment to guarantee a specified water quality for laboratory animal drinking water is nearing completion by a contractor and should be in place in the two primary animal housing buildings in the near future.

Invitations to present lectures, teaching assignments at local universities, and nominations to standing committees are accepted without rancor and are usually followed by active participation. The Chemistry Section is striving hard to establish a rapport with the various pertinent departments of the local universities in order to further enhance its research productivity and provide professional growth.

The Chemistry Section remains cognizant of the Institute safety program. A special hazardous chemical containment facility for the Section is nearing completion and should be operational in the near future. The facility will permit a variety of chemical operations involving hazardous chemicals to be conducted with the minimum exposure possible to laboratory personnel. The Section Office continues to cooperate with the Safety Office in design of specific chemical disposal procedures as needed. In addition, several Chemistry Section personnel devoted a considerable amount of time to resolution of the safe use of one of the Institute laboratory buildings which contains recirculating air systems. The Head of the Chemistry Section also serves as Chairman of the Safety Committee.

COMPARATIVE PATHOLOGY

During FY-78, the Comparative Pathology Section continued its four major functions: support of the NIEHS, Comparative Medicine Branch disease surveillance program (25%); support of Research Resources Program (Toxicology Bioassay Program, etc, 25%); support of Intramural Research (25%); and independent research (25%). Laboratory capability is maintained in histology, electron microscopy, anatomic pathology, and clinical pathology.

Accomplishments

1. Disease surveillance program - accomplishments are detailed in the reports of the Comparative Medicine Branch
2. Support of Intramural Research
 - a. A great deal of support was provided by the histology and EM labs in support of the Laboratory of Pulmonary Function and Toxicology. Lesser support was provided to the Laboratory of Mutagenesis and Laboratory of Pharmacology.
 - b. Anatomic Pathology
 - (1) Laboratory of Environmental Toxicology
 - (a) Drs. M. Peters and J. Kao - interpretation of lesions produced in the transplacental study of Methyl Butyl Ketone and Ethrane
 - (b) Dr. Staples - interpretation of natural and fetal lesions of mice produced by hexachlorobiphenyl
 - (c) Dr. Nawrot - interpretation of natural and fetal lesions produced by parazylene
 - (d) Dr. C. Kimmel - interpretation of the lesions produced by life time exposure to low levels of lead
 - (2) Laboratory of Pharmacology
 - (a) Dr. V. Van Ander - assistance in the interpretation of lesions produced in the isolated liver techniques
 - (3) Laboratory of Environmental Mutagenesis
 - (a) Dr. Frezza - Distribution of Yeast Cells in Different Organs of Mice
 - (4) Assistance in the interpretation of various other (smaller in terms of pathologists time) studies:

- (a) Dr. N. Carmichael - Studies on renal calculi in the mollusk
 - (b) Dr. L. Linnoila - assistance in learning the techniques of photomicrography
 - (c) Dr. I.P. Lee - review of sections of prostate from rats exposed to Freon F22
 - (d) Mr. G. McNichol - assistance in developing staining techniques for bone marrow studies
 - (e) Ms. W. Curley - assistance in the interpretation of lesions produced by DMH
 - (f) Several instances of CBCs for various scientists in the NIEHS
- (5) Several studies by investigators in other sections of EBCB were supported:
- (a) Dr. Lam - Inhalation toxicity of morpholine in the rat
 - (b) Dr. Tombropoulos - Inhalation toxicity of morpholine in the rabbit
 - (c) Dr. Kohli - Biochemical effects of pure isomers of HCB in the rat
 - (d) Dr. Goldstein - Evaluation of livers from rats used in MFO studies
 - (e) Dr. Hass - Study of biological effects of polar and non-polar fractions of FF-1
 - (f) Dr. McKinney - Study of biologic effects of various compounds used in a structure/activity experiment

3. Studies conducted in support of RRP targeted research included:

- a. The Comparative Toxicity of Technical vs Pure Pentachlorophenol in Dairy cattle
- b. Toxicity of Dibenzo-p-dioxin Isomers in Mice and Guinea Pigs
- c. Dose Response to the Carcinogens, 1-2-Dimethylhydrazine (DMH) and Methylazoxymethanol (MAM) in Rats
- d. Toxicity of Dibenzofuran Isomers In Guinea Pigs, Mice and Monkeys

- e. Participation in the design of a protocol for the study of various types of inhaled asbestos. Also, responsible for the interpretation of lesions produced in this study.
 - f. Assumption of the responsibility for the interpretations of lesions in the vinyl chloride cancer study being conducted under Contract.
4. Independent Research: Projects in this area included:
- a. LD₅₀ Study in Rats Exposed to Firemaster FF-1
 - b. Efforts to Enhance the Elimination of 2,3,7,8-Tetrachloro-dibenzo-p-dioxin from the Body of Rats
 - c. Studies on the Mechanism of Toxicity of 2,3,7,8-TCDD in Rats
 - d. A Study of the Toxicity of Japanese Rice (Yusho) Oil in Guinea Pigs
 - e. The Comparative Toxicity of Related Halogenated Polycyclic Hydrocarbons in Guinea Pigs
 - f. Assistance in protocol design and interpretation of lesions in studies of peritoneal macrophage function and effects of dietary protein levels in hamsters

INHALATION TOXICOLOGY

The program of the Inhalation Toxicology Section is composed of three parts: (1) Studies are conducted of compounds to which toxicologically significant exposure would be expected to be primarily by inhalation. Research is focused on expressions of toxicity at the levels of tissues, organs, and organ systems. Emphasis is placed on interactions among the cardiovascular system, the lungs and kidneys. (2) A principal effort of the Section is directed toward the advancement of inhalation technology. (3) Support is provided to intramural scientists outside the Section in the form of consultation, and collaboration, where appropriate, in matters of inhalation toxicology. The Section also allocates up to 50% of its small animal inhalation exposure chamber capacity to studies originating outside the Section as a service.

Inhalation Facility

The small animal inhalation facility is now operational under "computer" control. Data acquisition and chamber control are by means of an advanced electronic calculator rather than a minicomputer. Although somewhat slower and less versatile, a computer system controls 6 pairs of chambers; it is designed to accommodate 9. As more chemical analytical instruments are acquired, more pairs of chambers may be operated, or multiple compounds may be run in the same chamber(s). The compounds of interest

in the chamber are monitored with infrared gas analyzers whenever possible; provision has been made for the use of gas chromatographs as well. Data acquisition and control are more efficient when the infrared analyzers are used.

The design and assembly of the automated system has been a rewarding challenge. This project is expected to be complete by the end of 1978. At that point, the system should be operable by technicians with no more than occasional attention by professional personnel.

Manuscripts are in preparation describing the system in sufficient detail. The description will portray a unique feature of the system: the ability of an investigator to specify virtually any time varying profile, the shape of which is limited only by the "reaction time" of the system, rather than be wedded to one or two concentrations throughout the exposure, as is necessary when such a system is under manual control.

We have allotted one-half of our total exposure capacity to projects originating outside the Inhalation Toxicology Section. We have, throughout most of FY-78, had chambers up in support of such projects continually. With the automated system in operation our policy is one of having the facility operating at full capacity.

Complete exposure data summaries are provided the investigator both during the exposures and at the conclusion of each experiment. This includes records of the variables monitored that are relevant to the exposure such as concentration of the compound of interest, and chamber temperature and humidity. Furthermore, calibration data, quality control summaries, and estimates of the error in achieving the target profile are provided. These summaries take the form of printed tables and graphic summary of the exposure profile, generated by a peripheral plotter that is of a quality suitable for reproduction.

The facility is designed for exposure to organic vapors and cylinder gases, and oxides of nitrogen. We are not equipped for ozone, or solid or liquid aerosols.

Research

The Section research program is divided into four parts. They are studies of environmental contaminants on (1) the electromechanical activity of isolated, perfused whole hearts and other myocardial preparations in vitro, (2) the composition and function of pulmonary lavage and its constituents, and (3) pulmonary function in small animals. A fourth project serves as an umbrella for miscellaneous, related studies and is called Toxicology of Environmental Chemicals.

Morpholine was found to be relatively innocuous when administered IV or by inhalation to guinea pigs and rabbits in small quantities for brief periods. One remarkable observation was that the intravenous injection of boluses of micromolar quantities of morpholine in aqueous solution to guinea pigs caused a dose dependent, transient fall in mean arterial

blood pressure that was abolished by atropine. Exhaustive pharmacologic investigation and studies with various smooth muscle preparations from both rabbits and guinea pigs failed to identify the specific mechanism of action or probably principal site(s) of action. The tentative conclusion was drawn, however, that the response reflected an interaction of morpholine with muscarinic receptors rather than the myriad other mechanisms that could underlie such a phenomenon.

Pulmonary lavage is composed of glycolipids, glycoproteins, enzymes, macrophages, and other cells of pulmonary origin. Since the lung is the portal of entry for inhaled chemicals, it is logical to examine the composition and constituent function of the products of pulmonary lavage for evidence of toxic injury.

In the case of morpholine inhalation, the elevated α -N-acetylglucosaminidase and the decrease of binding by lung macrophages of wheat germ agglutinin which is specific for acetyl-d-glucosamine, agree very well. The high percentage of dead macrophages, the alterations of their surface membranes, and a reduction of lung microsomal P450 activity, indicate that the immune and detoxifying mechanisms of the lung remain impaired for at least 2 days post exposure.

One of the technical problems that has impeded progress in toxicological studies of the simpler organic amines is the difficulty of their analysis in biological material. We have developed a gas chromatographic method for such analyses and have proceeded to study the distribution of inhaled morpholine in the rabbit. As would be expected, the highest concentrations are found in the kidney, since the amine would be expected to be concentrated by the organic base tubular excretory mechanism. This a priori expectation was borne out in subsequent experiments in which labelled morpholine was shown to be accumulated by renal cortical slices in vitro. A complete characterization of the renal excretion of morpholine is underway and the intention is to compare it with its close relatives and structural analogs, diethanolamine, and 2-aminoethoxyethanol.

Whereas, the kidney begins extracting morpholine immediately, we discovered that 24 hours later it also appears in high concentrations in the bile. During exposure, significant quantities of morpholine are detectable in the blood and a variety of organs, including brain, but not adipose tissue. After 24 hours, significant quantities remain only in the kidney, spleen, and adrenals.

The first and last time that any detailed attempt was made to obtain classical toxicity data for morpholine was in 1939 and so we decided to re-evaluate these in consideration of the technological advances of the past 35 years. Our estimates of morpholine toxicity did not agree well with those of 1939, to the extent that the LC_{50} by inhalation was estimated to be much lower for rats and mice than previously reported.

The effects of morpholine on pulmonary function in the rats has been studied. Total lung capacity (TLC), residual volume (RV), alveolar volume (V_A), vital capacity (VC), lung weight (LW), and carbon monoxide

diffusing capacity (D_{LCO}) were determined. Morpholine was noted to decrease body weight gain, a variable that by itself, has been shown to affect certain of the variables measured. The interpretation of the data was complicated, i.e. morpholine effects on the variables of interest have to be separated from those secondary effects that were only related to the failure of normal weight gain. To solve this problem, functional tests were run on a separate set of non-exposed control animals. This sample allowed us to define the weight-"variable" relationship. This information combined with analysis of covariance enabled us to conclude that morpholine significantly decreased LW, D_{LCO} , and D_{LCO}/V_A , and significantly increased RV and TLC, all independently of the effect of morpholine on body weight. This project is being concluded and the pulmonary function testing research discontinued due to a manpower reduction in the Inhalation Toxicology Section.

IIT RESEARCH INSTITUTE - Chicago, Illinois 60616
(NIH-N01-ES-8-2115)

TITLE: Radiosynthesis of ^{14}C -2,3,7,8-Tetrachlorodibenzofuran (TCDF)

CONTRACTOR'S PROJECT DIRECTOR: Allan P. Gray, Ph.D.

PROJECT OFFICER (NIEHS): J. D. McKinney, Ph.D., Supervisory Research
Chemist, Chemistry Section, EBCB

DATE CONTRACT INITIATED: March 27, 1978

CURRENT LEVEL (150 days): \$30,000

PROJECT DESCRIPTION

OBJECTIVES: Synthesis of 10 millicuries of ^{14}C -radiolabeled 2,3,7,8-TCDF at the highest specific activity reasonably achievable but not less than 50 millicuries/millimole and with a chemical and radiochemical purity greater than 98 percent.

METHODS EMPLOYED: Usual radiosynthetic techniques.

MAJOR FINDINGS AND PROPOSED COURSE: The compound, 2,3,7,8-TCDF has been previously synthesized unlabeled by IIT; however, its synthesis as a radiolabeled compound will require many different considerations and development of methodology. In this regard, two aborted attempts by the proposed contractor to prepare this compound labeled has shown that the selection of which precursor compound should contain the label and the reaction intermediates involved are critical in the radiosynthesis. This result was unexpected and appears to be related to the high specific activity and accompanying radiochemistry of "hot" free radical intermediates. Therefore, it will be necessary to repeat the entire reaction scheme using an entirely different and more radiochemically reliable route, viz. via O-nitro-chlorobenzene. The proposed contractor has now gained considerably more experience in carrying out the entire basic reaction sequence.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The chlorinated dibenzofurans occur as contaminants in commercially produced polychlorinated biphenyl (PCBs). Currently there is little known about the toxicity and biological fate of these compounds. It is presently assumed that the 2,3,7,8-TCDF is the most toxic member of the class of chemicals. Studies at NIEHS have shown it to be highly toxic for the guinea pig, but markedly less toxic for mice and rats. Studies on these species' difference in regard to variation in adsorption, metabolism, distribution and excretion are urgently needed. Such studies are most rapidly and conveniently done using radiolabeled compounds.

SCIENCE APPLICATIONS, INC. - La Jolla, California 92038
(NIH-NO1-ES-8-2105)

TITLE: Analysis of Total Organic Chlorine and Bromine Residues in
Human Body Fluids and Tissues

CONTRACTOR'S PROJECT DIRECTOR: J. Reed

PROJECT OFFICER (NIEHS): J. D. McKinney, Ph.D., Supervisory Research
Chemist, Chemistry Section, EBCB

DATE CONTRACT INITIATED: December 20, 1977

CURRENT LEVEL (3 years): \$169,712

PROJECT DESCRIPTION

OBJECTIVES: Analysis of 750 to 1,000 samples per year of breast milk, formula, blood serum, and tissue for total organic chlorine (TOCl) and bromine (TOBr) of whole fluid or wet tissue or for total soluble organic chlorine (TSOCl) and bromine (TSOBr) content of portions of extractable lipids. The desired detection thresholds range from 5-20 ng chlorine and 0.1-15 ng bromine/l gm milk.

METHODS EMPLOYED: Somogyi method of desalting followed by standard methods of sample preparation for neutron activation analysis.

MAJOR FINDINGS AND PROPOSED COURSE: The contractors major effort to date has been completion of literature searches, performance of neutron activation analysis on blanks, reagents, and solvents and development of a modification of the Somogyi procedure. Problems have been encountered in obtainment of deionized water and certain solvents with low hazard levels of chlorine and bromine and in the determination of TOSCl in placenta tissue lipid extracts as well as perfection of the milk desalting procedure. The contractor has gained insight into these problems during the first three months of the program; however, additional effort is still required. We propose to continue this work to perfect necessary procedures and to develop methods for the analysis of "pooled" milk, blood and tissue and to develop and perfect the coordination necessary between the two contractors.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Obtainment of accurate and reliable results that lead to meaningful interpretation of the transfer of PCB's and DDE from mother to child through placental tissue membranes or through breast milk requires the separation of inorganic bound chloride and bromine from inorganic chlorides and bromides prior to neutron activation analysis for TOCl, TOBr, TSOCl and TSOBr. This study integrates contract NIH-NO1-ES-7-2141 to provide a mass balance which indicates whether all the halides are accounted for by the PCB's and DDE. In this manner, the results of the contract for the analysis of PCB's and

DDE in human body fluids and tissues can be validated. This study will help resolve the important epidemiological effects of possible transplacental and breast milk transfer of environmental contaminants from mothers to babies in the United States.

WARF INSTITUTE, INC. - Madison, Wisconsin 60616
(NIH-N01-ES-7-2141)

TITLE: Analysis of PCB's and DDE in Human Body Fluids and Tissue

CONTRACTOR'S PROJECT DIRECTOR: D. L. Hughes

PROJECT OFFICER (NIEHS): J. D. McKinney, Ph.D., Supervisory Research
Chemist, Chemistry Section, EBCB

DATE CONTRACT INITIATED: September 30, 1977

CURRENT LEVEL (3 years): \$267,656

PROJECT DESCRIPTION

OBJECTIVES: Analysis of 750 to 1,000 samples per year of breast milk, formula, blood serum and placental tissue for polychlorinated biphenyls (PCB's) and 1,1-bis(p-chlorobiphenyl)-2,2-dichloroethane (DDE). The desired detection thresholds range from 0.5 to 50 ppb depending on the type of sample.

METHODS EMPLOYED: Gas liquid electron capture chromatography and usual sample preparation, clean-up, extraction and lipid determination techniques.

MAJOR FINDINGS AND PROPOSED COURSE: This contract provides for development of the methodology for the analysis and the generation of suitable and reliable analytical data on PCB's and DDE in human body fluids and tissues, and is interfaced with contract (NIH-N01-ES-8-2105) for the analysis of organic chlorine and bromine in same media. Contract NIH-N01-ES-8-2105 serves as a check and validation of the significance of the results. The major effort of the contractor to date has been development of procedures for the analysis of placental tissue via a soxhlet extraction followed by column chromatography and Florisil using petroleum ether. Good recoveries have resulted from this method. Preliminary gas chromatographic analysis of blank and spiked milk samples using two different columns yielded encouraging results; however, further testing is necessary. We propose to continue method development on "pooled" milk, blood, and placental tissue to refine and perfect procedures and coordinate techniques with the other contract group.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The possible effect of transplacental and breast milk transfer of environmental contaminants from mothers to babies is an important and recurring epidemiological question. It has been shown that environmental contamination of breast milk occurs in the United States. Organohalogen pesticides and the polychlorinated biphenyls (PCB's) are widespread contaminants of breast milk. This study provides an integral part of an overall study of the possible widespread contamination of breast milk by environmental contaminants such as PCB's and their effects on infant development and health.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30003-06 EBCB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Development of Analytical Methodology		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: P.W. Albro OTHER: J.R. Hass K. Chae K. Kohli	Research Chemist Senior Staff Fellow Staff Fellow Visiting Fellow	EBCB NIEHS EBCB NIEHS EBCB NIEHS EBCB NIEHS
COOPERATING UNITS (if any) None		
LAB/BRANCH Environmental Biology and Chemistry Branch		
SECTION Chemistry Section		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.50	PROFESSIONAL: 0.25	OTHER: 0.25
CHECK APPROPRIATE BOX(ES)		
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER		
☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
This general project area has as its objective, to develop and refine methodology for the <u>quantitative and qualitative determination</u> of compounds and classes of compounds of general interest to the Institute and specific interest to individual investigators. Recent work has emphasized development and validation of methods for (a) <u>quantitative and reproducible extraction and clean-up of halogenated aromatic compounds</u> from biological materials for subsequent GC-MS or immunoassay, and (b) <u>analysis of the spontaneous breakdown products of unstable xenobiotics.</u>		

PROJECT DESCRIPTION

METHODS EMPLOYED: The analytic methods will generally be developed using gas chromatography, thin-layer and column chromatography, fluorometry, spectrophotometry (IR, UV, Visible), mass spectrometry and isotopic methods. Other special methods will be employed where necessary or as other instrumentation becomes available.

MAJOR FINDINGS AND PROPOSED COURSE: Methods for the quantitative extraction of halogenated aromatics from liver, skin, whole blood, adipose and milk have been evaluated and standardized. Clean-up procedures for PCB's, PBB's, dibenzodioxins and furans, and quadphenyls in such extracts have been reduced to 2-3 steps. Previously, unsuspected or unconfirmed sources of contamination or loss have been identified. Five types of chromatographic alumina and two activation procedures have been studied, leading to the selection of acidic alumina (e.g. Merck 1078) activated at 130° as the most advantageous for use in clean-up of biological extracts. A procedure for isotope dilution assay has been developed, permitting the determination of "total PCB content" of tissues. The pathway of decomposition of procabazine has been elucidated. Methodologies will continue to be developed as needed.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Successful development of the analytical methodology in the areas delineated above will accelerate the successful elaboration of a number of metabolic and degradation studies in progress in the Chemistry Section as well as be of utility for other studies within the Institute.

PUBLICATIONS

Albro, P.W., and Corbett, B.J.: Extraction and clean-up of animal tissues for subsequent determination of mixtures of chlorinated dibenzo-p-dioxins and dibenzofurans. Chemosphere, 7: 381-385, 1977.

Albro, P.W., Chae, K., Hass, J.R., Kohli, K., Harvan, D., Haseman, J., and Clemmer, T.: Newer methods in the analysis of polychlorinated biphenyls. Environ. Health Perspect. 20: 246, 1977. (abstract)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30005-10 EBCB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Toxicity of Dibenzodioxins		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	J. A. Moore	Supervisory Veterinary Medical Officer EBCB NIEHS
OTHER:	E. E. McConnell	Veterinary Pathologist EBCB NIEHS
	M. W. Harris	Biological Laboratory Technician EBCB NIEHS
COOPERATING UNITS (if any) K. Chae, Ph.D., Chemistry Section, EBCB		
LAB/BRANCH Environmental Biology and Chemistry Branch		
SECTION Comparative Biology Section		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.2	OTHER: 0.4
CHECK APPROPRIATE BOX(ES)		
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER		
☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this study is to evaluate various <u>dibenzodioxins</u> with regard to the following parameters (1) <u>dose response</u> ; (2) <u>lethal dose</u> ; (3) <u>clinical signs</u> ; (4) <u>clinical pathology</u> ; and (5) <u>anatomic pathology</u> in <u>guinea pigs</u> and <u>mice</u> .		

PROJECT DESCRIPTION

METHODS EMPLOYED: Mice, and guinea pigs were given single or multiple doses of various dibenzodioxins (see below) orally in order to study the toxicologic, pathologic and clinical pathologic effects of the intoxication. Standard techniques were used in order to evaluate these effects.

MAJOR FINDINGS AND PROPOSED COURSE: The toxicopathology of the above compounds was studied in C57B1/6gh (J67) mice and Hartley guinea pigs. The single oral LD₅₀ was determined for these compounds in both species of animals (Table 1). 2,3,7,8-TCDD was found more toxic than any of the other isomers. However, 1,2,3,7,8-PCDD was almost as toxic. The lesions produced by all compounds were similar in a given species. Thymic and splenic atrophy, toxic hepatitis, vascular lesions (edema and hemorrhage) and serum protein dyscrasias were the prominent features in mice. In contrast, thymic, testicular and bone marrow atrophy along with adrenal cortical hemorrhage and proliferation of the renal pelvic epithelium were more characteristically found in guinea pigs. This project is to be continued.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Chlorinated dibenzodioxins may occur in association with many useful chlorinated cyclic compounds. The symmetrical tetrachloro isomer is one of the most toxic chemicals known. The pattern of toxicity associated with most of the isomers has not been determined. The interactions of isomers as they relate to toxicity is unknown. These facts must be determined if assessment of the environmental health implication of products which contain dibenzodioxin isomers as contaminants are to be made.

PUBLICATIONS

McConnell, E.E., Moore, J.A., Haseman, J.K., and Harris, M.W.: The Comparative Toxicity of Chlorinated Dibenzo-p-dioxin Isomers in Mice and Guinea Pigs. Toxicol. Appl. Pharmacol. 44: 335-356, 1978.

McConnell, E.E., Moore, J.A., Harris, M.W., and Haseman, J.K.: Toxicity of Chlorinated Dibenzo-p-dioxins in Mice, Guinea Pigs, and Rhesus Monkeys. Environ. Health Persp. 20: 245, 1977.

McConnell, E.E., and Moore, J.A.: The Toxicopathology of TCDD. In Cattabeni, S (Ed.): Proceed. Workshop on TCDD. Holliswood, New York, Spectrum Publishing, 1978, pp. 137-141.

McConnell, E.E. and Talley, F.A.: Intracytoplasmic Hyaline Globules in the Adrenal Medulla of Laboratory Animals. Vet. Path. 14: 435-440, 1977.

Moore, J.A.: Toxicity of 2,3,7,8-Tetrachlorodibenzo-para-dioxin. Ecol. Bull. 27: 134-144, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30012-05 EBCB																				
PERIOD COVERED October 1, 1977 to September 30, 1978																						
TITLE OF PROJECT (80 characters or less) Effects of Pure Isomers of Hexachlorobiphenyls on Lipid Metabolism and Cell Structure																						
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">K. Kohli</td> <td style="width: 35%;">Visiting Fellow</td> <td style="width: 15%;">EBCB NIEHS</td> </tr> <tr> <td>OTHER:</td> <td>P. Albro</td> <td>Research Chemist</td> <td>EBCB NIEHS</td> </tr> <tr> <td></td> <td>B. Gupta</td> <td>Veterinary Medical Officer</td> <td>EBCB NIEHS</td> </tr> <tr> <td></td> <td>H. Mukhtar</td> <td>Visiting Associate</td> <td>LP NIEHS</td> </tr> <tr> <td></td> <td>J. McKinney</td> <td>Supervisory Research Chemist</td> <td>EBCB NIEHS</td> </tr> </table>			PI:	K. Kohli	Visiting Fellow	EBCB NIEHS	OTHER:	P. Albro	Research Chemist	EBCB NIEHS		B. Gupta	Veterinary Medical Officer	EBCB NIEHS		H. Mukhtar	Visiting Associate	LP NIEHS		J. McKinney	Supervisory Research Chemist	EBCB NIEHS
PI:	K. Kohli	Visiting Fellow	EBCB NIEHS																			
OTHER:	P. Albro	Research Chemist	EBCB NIEHS																			
	B. Gupta	Veterinary Medical Officer	EBCB NIEHS																			
	H. Mukhtar	Visiting Associate	LP NIEHS																			
	J. McKinney	Supervisory Research Chemist	EBCB NIEHS																			
COOPERATING UNITS (if any) Animal Husbandry Section, EBCB and Marine Pharmacology Section, LP																						
LAB/BRANCH Environmental Biology and Chemistry Branch																						
SECTION Chemistry Section																						
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																						
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:																				
0.9	0.9	0.0																				
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (a1) MINORS ☐ (a2) INTERVIEWS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER																						
SUMMARY OF WORK (200 words or less - underline keywords) The effects of single oral doses of 3,3',4,4',5,5'-; 2,2',4,4',5,5'-; and 2,2',3,3',5,5'-hexachlorobiphenyls on <u>lipid metabolism</u> and <u>cell structure</u>, as well as on <u>induction</u> of various enzyme activities in the livers of rats have been studied. <u>Structure-dependent effects</u> have been observed, and effects of 2,2',3,3',5,5'-hexachlorobiphenyl demonstrate that <u>p-substitution</u> is not essential for biological activity.																						

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard lipid chemistry analytical methods, oral feeding of PCBs in corn oil by gavage, routine enzyme assays, histologic examination.

MAJOR FINDINGS AND PROPOSED COURSE: The effects of a single oral dose (50 mg/kg body weight) of 3,4,5,3',4',5'-hexachlorobiphenyl (HCB), 2,4,5,2',4',5'-HCB and 2,3,5,2',3',5'-HCB for a period of 72 hr have been studied in the male rat. 3,4,5,3',4',5'-HCB caused necrosis of thymocytes where as other isomers did not. 3,4,5,3',4',5'-HCB caused marked pathological changes in the liver which was not affected so markedly by 2,4,5,2',4',5'-HCB and 2,3,5,2',3',5'-HCB. Total lipid content was increased by all the isomers studied. 3,4,5,3',4',5'-HCB had more pronounced effects on total lipid content than the other two isomers. Lipid pattern in the hepatocytes obtained from rats treated with 3,4,5,3',4',5'-HCB was pericentral and midzonal in hepatocytes obtained from the rats treated with the other two isomers. Analyses of various lipid fractions showed that triacylglycerols were increased seven fold on feeding of 3,4,5,3',4',5'-HCB, whereas phospholipids were increased slightly on feeding of 2,4,5,2',4',5'-HCB or 2,3,5,2',3',5'-HCB. The latter two isomers did not affect the level of triacylglycerols. 3,4,5,3',4',5'-HCB also increased the level of total and esterified cholesterol, which was not affected by the other two isomers studied. These results show that the fatty livers caused by 3,4,5,3',4',5'-HCB were qualitatively and quantitatively different than the other two isomers studied. For the first time a hexachlorobiphenyl not chlorinated in the para position, 2,3,5,2',3',5'-HCB has been shown to be biologically active. The magnitude of the effects of 2,3,5,2',3',5'-HCB on cell structure and phospholipid content was the equivalent to those caused by 2,4,5,2',4',5'-HCB. Chlorination at para position may not be necessary for a chlorobiphenyl isomer to be biologically active. Total stereoelectronic properties of the isomers may be more important in determination of structure-activity relationships. Future work will include identification of the enzyme activities affected by treatment with PCBs and responsible for alterations in lipid composition.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

These studies, and related studies of structure-activity relationships for the family of PCB compounds will contribute to our ability to predict the hazards of previously untested PCB-like compounds. This is desirable because of the ubiquitous nature of PCB pollution of the environment and the variety of PCB isomers and related compounds involved.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30015-04 EBCB						
PERIOD COVERED October 1, 1977 to September 30, 1978								
TITLE OF PROJECT (80 characters or less) Studies in Chemical Ionization Mass Spectrometry								
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT								
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: J. R. Hass</td> <td style="width: 33%;">Senior Staff Fellow</td> <td style="width: 33%;">EBCB NIEHS</td> </tr> <tr> <td>OTHER: M. M. Bursey</td> <td>Visiting Scientist</td> <td>EBCB NIEHS</td> </tr> </table>			PI: J. R. Hass	Senior Staff Fellow	EBCB NIEHS	OTHER: M. M. Bursey	Visiting Scientist	EBCB NIEHS
PI: J. R. Hass	Senior Staff Fellow	EBCB NIEHS						
OTHER: M. M. Bursey	Visiting Scientist	EBCB NIEHS						
COOPERATING UNITS (if any) None								
LAB/BRANCH Environmental Biology and Chemistry Branch								
SECTION Chemistry Section								
INSTITUTE AND LOCATION NIEHS, NTH, Research Triangle Park, North Carolina 27709								
TOTAL MANYEARS: 1.5	PROFESSIONAL: 0.5	OTHER: 1.0						
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS								
SUMMARY OF WORK (200 words or less - underline keywords) The <u>negative ion chemical ionization mass spectra</u> of 14 typical <u>organo-phosphorus pesticides</u> , <u>25 nitriles</u> , and all the <u>chlorinated anisoles</u> (as models for the fragmentation patterns of polychlorinated diphenyl ethers and polychlorinated dibenzodioxins) were interpreted with respect to their analysis in environmental samples.								

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard gas chromatography/mass spectrometry techniques, negative ion chemical ionization mass spectrometry.

MAJOR FINDINGS AND PROPOSED COURSE: Three classes of compounds were studied by negative ion chemical ionization mass spectrometry: organophosphorus pesticides, nitriles, and polychlorinated anisoles. The last named were studied as models for polychlorinated dioxins and diphenyl ethers.

The organophosphorus esters were shown to have characteristic fragmentations sensitive to the nature of the substitution pattern and, in representative cases, to be detectable at lower limits than with conventional positive ion chemical ionization mass spectrometry. Correlations of substitution pattern with specific reactions observed in negative ion chemical ionization mass spectrometry were made for the polychlorinated anisoles; these will act as guides to understanding the specificity of certain losses and ion-molecule reactions found in the spectra of dioxins and polychlorinated diphenyl ethers. The negative ion chemical ionization mass spectra of nitriles were shown to depend on the site of nitrile substitution and other structural features; several unprecedented types of ion-molecule reactions were observed.

This project will be extended by the study of the CI mass spectra of other environmental hazards.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Negative ion chemical ionization mass spectrometry was shown to permit analysis of several classes of environmental contaminants at lower levels than previously possible. For others, a better understanding of the interpretation of the mass spectra will lead to better methods of structural identification of organic contaminants at the ppb level. Chemical ionization techniques have provided information complementary to that obtained using electron impact ionization methods. The methods employed in this project allows one to gather further information concerning the nature of an unknown sample.

PUBLICATIONS

Busch, K.L., Bursey, M.M., Hass, J.R., and Sovocool, G.W.: Comparison of five ionization methods for producing mass spectra of typical organophosphorus pesticides. Appl. Spectrosc. (in press)

Busch, K.L., Hass, J.R., and Bursey, M.M.: The gas enhanced negative ion mass spectra of polychloroanisoles. Org. Mass Spectrom. (in press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30018-05
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Ester Hydrolases at the Environmental Interface		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: P. W. Albro OTHER: None Research Chemist EBCB NIEHS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Environmental Biology and Chemistry Branch		
SECTION Chemistry Section		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.0	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER		
☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The objective of this project is to evaluate the capabilities of various tissue lipases and esterases as early mediators in the interactions of animals with <u>xenobiotics</u> containing <u>ester linkages</u>. These enzymes are also used as models in studies of <u>protein-ligand interactions</u>. Recent studies have led to partial characterization of the <u>enzyme</u> in <u>human plasma</u> responsible for the hydrolysis of <u>phthalate diesters</u> (from blood bag plastics) to the more soluble monoesters. This esterase has been found to be distinguishable from both triglyceride lipase and lipoprotein lipase.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Most studies will involve the rat, but significant findings will be checked in mice, hamsters, guinea pigs and rabbits. Lung, gastrointestinal, and skin enzymes will be studied, using techniques generally described previously (Biochim. Biophys. Acta, 360: 380, 1973). Test substances will initially include: (1) esters of 2,4-D and 2,4,5-T, (2) phthalate plasticizers, (3) detergents such as Tween 20, Tween 80, (4) aromatic rubrefacients such as methyl paraben, menthyl salicylate, (5) volatile solvents such as ethyl acetate, butyl acetate, (6) carbamates.

MAJOR FINDINGS AND PROPOSED COURSE: Planned studies of protein-lipid interactions using nonspecific lipase from rat pancreas were delayed since no bids were received on our request for a large-scale purification of the enzyme. The heat-sensitive nonspecific lipase in rat plasma appears to be functionally identical to that in (outdated) human plasma. It is active even at -20°, and catalyzes the hydrolysis of lipoprotein-associated lipid esters. It is more active against phthalates than against triglycerides, and is responsible for the accumulation of mono-ethylhexyl phthalate in blood or plasma stored in plastic blood bags. We probably will not attempt to isolate this enzyme, but rather will begin studies of analogous enzymes in skin.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Relatively little is known about non-specific lipase activity, since the enzyme(s) has(have) never previously been purified. Although a great deal is known about lung esterases, very little is known about lung lipases (hydrolases for insoluble esters). Virtually nothing is known about ester hydrolases in skin. These enzymes may either protect against some hazardous compounds by converting them to less easily absorbed, more polar materials, or increase the hazard of other compounds when the acid (or alcohol) is more toxic than the ester. A second anticipated result of this project should be a better understanding of how enzymes in general are able to bind to and react with water-insoluble compounds.

PUBLICATIONS

Peck, C.C., Albro, P.W., Odom, D.G., and Hass, J.R.: Plasticizers in stored blood. in Proc. LAIR Symp. Microaggregates; Exptl. and Clin. Aspects, Dr. Louis Kozloff (ed.), W.H. Green, Inc., Conf. held summer 1977.

Albro, P.W., and Corbett, J.T.: Distribution of di- and mono-(2-ethylhexyl) phthalate in human plasma. Transfusion (in press).

Peck, C.C., Odom, D.G., Friedman, H.I., Albro, P.W., Hass, J.R., Brady, J.T., and Jess, D.A.: Di-2-ethylhexyl phthalate (DEHP) and mono-2-ethylhexyl phthalate (MEHP) accumulation in whole blood and red cell concentrates. Transfusion (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30020-07 EBCB						
PERIOD COVERED October 1, 1977 to September 30, 1978								
TITLE OF PROJECT (80 characters or less) Transport and Metabolism of Phthalate Esters								
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%;"> <tr> <td style="width: 33%;">PI: P. W. Albro</td> <td style="width: 33%;">Research Chemist</td> <td style="width: 33%;">EBCB NIEHS</td> </tr> <tr> <td>OTHER: J. R. Hass</td> <td>Senior Staff Fellow</td> <td>EBCB NIEHS</td> </tr> </table>			PI: P. W. Albro	Research Chemist	EBCB NIEHS	OTHER: J. R. Hass	Senior Staff Fellow	EBCB NIEHS
PI: P. W. Albro	Research Chemist	EBCB NIEHS						
OTHER: J. R. Hass	Senior Staff Fellow	EBCB NIEHS						
COOPERATING UNITS (if any) C. C. Peck, M.D., LTC, MC, LAIR								
LAB/BRANCH Environmental Biology and Chemistry Branch								
SECTION Chemistry Section								
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709								
TOTAL MANYEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0.0						
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS								
SUMMARY OF WORK (200 words or less - underline keywords) <u>CID processes have been used to confirm the identification of ten metabolites of diethylhexyl phthalate (DEHP), which have been isolated in small quantities as reference standards for metabolism studies. Plans are underway (at LAIR) to use ¹³C-labeled DEHP for studies of plasticizer pharmacokinetics in man. The role of NIEHS will be solely to analyze plasma and urine samples provided by LAIR.</u>								

PROJECT DESCRIPTION

METHODS EMPLOYED: Chromatography, spectrophotometry, mass spectrometry, isotopic methods, standard enzymology techniques.

MAJOR FINDINGS AND PROPOSED COURSE: The previously isolated (rat) metabolites of DEHP have been unequivocally identified by mass spec CID methods. The proposed study of ^{13}C -phthalate pharmacokinetics in man is delayed while the necessary permission to use human subjects is obtained by LAIR.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The phthalic acid esters are important industrial chemicals employed with extensive utility, primarily as plasticizers. Recent evidence of their migration into human tissues as well as their increasing occurrence in the ecology have been cited. Knowledge of the consequences of chronic or subacute ingestion, absorption and/or inhalation is essential for assessing the parameters of potential hazard of this environmental agent.

PUBLICATIONS

Peck, C.C., Albro, P.W., Hass, J.R., Odom, D.G., Barrett, B.B., and Bailey, J.: Metabolism and excretion of the plasticizer di-(2-ethylhexyl) phthalate in man. Clin. Res., 26: 101A, 1978. (abstract)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30034-03-EBCB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Chemistry of Chlorinated Aromatic Compounds and Their Environmental Transformation Products		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: L. A. Levy OTHER: O. Hernandez R. Cox	Research Chemist Visiting Associate Research Chemist	EBCB NIEHS EBCB NIEHS EBCB NIEHS
COOPERATING UNITS (if any) None		
LAB/BRANCH Environmental Biology and Chemistry Branch		
SECTION Chemistry Section		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 1.2	PROFESSIONAL: 1.2	OTHER: 0.25
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The development of rational synthetic routes to halogenated naphthalenes, chlorinated biphenyls and especially the assumed reactive intermediate arene oxides have been investigated. Models appropriate to the study of the chemical and physical properties of these classes of compounds as potential persistent environmental agents have been prepared.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Synthetic techniques, organometallic reagents and catalysis, high pressure reactions, photochemical processes, functional group transformations. Mass spectroscopy, other spectroscopic methods (IR, NMR), chromatography (column, glc, liquid).

MAJOR FINDINGS AND PROPOSED COURSE: The Diels-Alder cycloaddition reaction has been utilized in two different ways to generate the naphthalene nucleus with substituents appropriate to further transformation. Both of these approaches utilized a ortho xylylene synthon, generated from different precursors, which was allowed to undergo cycloaddition with different dieneophiles to produce the naphthalene nucleus. These derivatives have led to chlorinated and brominated naphthalenes as well as intermediates with the potential to be further elaborated to other halogenated naphthalenes and their possible metabolites.

Further developmental work on the problem of the synthesis of biphenyl and naphthalene oxides has provided methods suitable for the large scale production of intermediates appropriate for the final synthetic steps.

Some chlorinated phenanthrenes have been prepared. This was done to consider the question of toxicity as a function of structure and as models to further probe the nature of the interactions of ortho-ortho prime chloro substituents in biphenyls. The NMR spectra of these derivatives have been determined as a further aide in understanding the nature of the intramolecular interactions present in the biphenyl structural unit. The structure of some of these compounds in the solid state is to be examined by X-ray crystallography to further refine the molecular parameters. Some questions about the fragmentation observed in the mass spectra of PCB's which were raised in earlier work may be answered from the study of these phenanthrene derivatives, which incorporate a constrained biphenyl unit within their structure.

It is proposed to continue the synthesis of individual isomers of halogenated naphthalenes as required and especially their potential or actual metabolites such as arene oxides and hydroxylated derivatives. Continuing attention is to be focused on the use of the cycloaddition reaction and associated sequence mentioned above. The preparation of appropriate models to more fully explore the chemical and spectral characteristics of the molecules will be carried out as time permits.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The commercial PCB's and halogenated naphthalenes are known to be complex mixtures and are recognized to be of major environmental importance due to their widespread ecological distribution and their concentration in the food chain. Furthermore, chlorinated dibenzofuran impurities found along with PCB and/or produced from PCB by natural processes are known to be more toxic. The availability of the unique isomers of these classes of compounds

and their probable metabolites would permit more definitive biological and toxicological studies.

PUBLICATIONS

Levy, L.A.: Chemistry and synthesis of 2,3,6,7-tetrasubstituted naphthalenes. Proc. of the 175th ACS Meeting, March 1978. (abstract)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30035-03 EBCB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Chemical Techniques and Spectroscopic Methods		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: OTHER:	J. D. McKinney P. A. Albro J. R. Hass R. H. Cox K. C. Chae L. A. Levy C. E. Parker D. B. Walters	Supervisory Research Chemist Research Chemist Senior Staff Fellow Research Chemist Staff Fellow Research Chemist Research Chemist Chemical Management Specialist EBCB NIEHS EBCB NIEHS EBCB NIEHS EBCB NIEHS EBCB NIEHS EBCB NIEHS EBCB NIEHS EBCB NIEHS
COOPERATING UNITS (if any) NCI Chemical Repository. Others as needed.		
LAB/BRANCH Environmental Biology and Chemistry Branch		
SECTION Chemistry Section		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 3.1	PROFESSIONAL: 1.6	OTHER: 1.5
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) This general project has as its objective to employ <u>chemical techniques</u> , both analytical and synthetic, and <u>spectroscopic methods</u> , in particular gas chromatography/mass spectrometry (GC/MS), nuclear magnetic resonance spectroscopy (NMR) and high pressure liquid chromatography (HPLC), to the preparation, determination and characterization of compounds and classes of compounds of general interest to the Institute and specific interest to individual investigators. In general compounds covered by this study will include those for which adequate techniques and methods are already available or can be adapted. <u>Chemical management services</u> are also performed in support of various research programs.		

PROJECT DESCRIPTION

METHODS EMPLOYED: The analytical methods emphasized in this study are GC/MS, NMR or fourier transform NMR and HPLC. A variety of synthetic techniques are employed. Other special methods will be employed where necessary or as other instrumentation becomes available.

MAJOR FINDINGS AND PROPOSED COURSE: The GC/MS facility made the largest number (over 2300) of sample runs for analysis. About 39% of these was for routine service request, and the remaining in collaborative support of various biological studies and instrumental and method development research. Only a small percent of the total number of samples run were performed using the high resolution mass spectrometer with the data system. This number is expected to increase as this relatedly new system continues to undergo stabilization.

Procedures have been established to facilitate the use of Chemistry Section HPLC equipment by other Institute staff. Over 230 analyses have been performed through scheduling the use of this equipment.

The NMR facility handled a number of different samples including a variety of chemical and biological metabolites and in many observed both ^1H and ^{13}C nuclei using ^1H decoupling and fourier transform (FT) techniques. Most of these samples were generated from other projects within the section. The use of the NMR facility by Institute staff outside of Chemistry is expected to increase with the addition of research programs requiring a greater degree of mechanistic study at the molecular level.

The more difficult or involved synthetic and/or preparative requests included aborted attempts to prepare the catechol derivative of diethylstilbestrol, synthesis of ^{14}C -labeled 1,2-methylene (^{14}C) dioxy-4-propylbenzene, selected brominated biphenyl and unsymmetrical chlorinated biphenyl isomers, and O-carboxymethyl estrone.

A number of other requests were made for analytical service which required specific methods. These ranged from specific analyses for DNA, lipid classes and fatty acid compositions, specific isomer content, metabolites and purity and radiopurity of unlabeled and labeled research chemicals to syntheses, biosyntheses and determination of reaction rates and reactivity profiles. These types of analyses totaled over 800 samples of which the majority were for DNA analysis.

Chemical management services facilitated the procurement, storage, dispensal, and where necessary, disposal of specialty research chemicals.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The preparation, determination and characterization of various compounds and compound classes of general interest to the Institute will facilitate the successful elaboration of their chemistry and biochemistry and permit more definitive biological and toxicological studies.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30038-04 EBCB												
PERIOD COVERED October 1, 1977 to September 30, 1978														
TITLE OF PROJECT (80 characters or less) Toxicity of Dibenzofurans														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">J.A. Moore</td> <td style="width: 40%;">Supervisory Veterinary Medical Officer</td> <td style="width: 10%;">EBCB NIEHS</td> </tr> <tr> <td>OTHER:</td> <td>E.E. McConnell</td> <td>Veterinary Pathologist</td> <td>EBCB NIEHS</td> </tr> <tr> <td></td> <td>M.W. Harris</td> <td>Biological Laboratory Technician</td> <td>EBCB NIEHS</td> </tr> </table>			PI:	J.A. Moore	Supervisory Veterinary Medical Officer	EBCB NIEHS	OTHER:	E.E. McConnell	Veterinary Pathologist	EBCB NIEHS		M.W. Harris	Biological Laboratory Technician	EBCB NIEHS
PI:	J.A. Moore	Supervisory Veterinary Medical Officer	EBCB NIEHS											
OTHER:	E.E. McConnell	Veterinary Pathologist	EBCB NIEHS											
	M.W. Harris	Biological Laboratory Technician	EBCB NIEHS											
COOPERATING UNITS (if any) K. Chae, Ph.D., Chemistry Section, EBCB Hazleton Research Laboratories, Vienna, VA														
LAB/BRANCH Environmental Biology and Chemistry Branch														
SECTION Comparative Biology Section														
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709														
TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.1	OTHER: 0.1												
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this study is to evaluate various dibenzofurans with regard to the following parameters (1) <u>Dose response</u> ; (2) <u>Lethal dose</u> ; (3) <u>Clinical signs</u> ; (4) <u>Clinical Pathology</u> ; and (5) <u>Anatomic Pathology</u> in <u>guinea pigs</u> , <u>mice</u> and <u>monkeys</u> .														

PROJECT DESCRIPTION

METHODS EMPLOYED: Male guinea pigs and/or mice were given single or multiple doses of various dibenzofurans [2, 3, 7, 8-tetrachlorodibenzofuran (TCDF), 2, 3, 7, 8-tetrabromodibenzofuran (TBDF), and 2, 3, 7, 8-pentachlorodibenzofuran (PCDF)] orally. Rhesus monkeys were given single oral doses of TCDF. Standard techniques were used to evaluate the objectives.

MAJOR FINDINGS AND PROPOSED COURSE: These compounds appear to be extremely toxic with the LD₅₀ in guinea pigs being 5-10 µg/kg for TCDF and 3-10 µg/kg for PCDF or about 3 to 5 times less toxic than their dioxin counterpart. The brominated form (TBDF) was of about the same toxicity (3-10 µg/kg) as TCDF. The LD₅₀ of TCDF in Rhesus monkeys was 1000 µg/kg. The lesions produced by these compounds were similar in a given species of animal. However, a distinct difference was noted between species. Thymic and splenic atrophy were noted in all species. Few lesions were found in mice because it was impossible to give a high enough single oral dose (> 6000 µg/kg) to cause significant toxicity. Testicular and bone marrow atrophy along with adrenal cortical hemorrhage and hyperplasia of the renal pelvic epithelium were more characteristically found in guinea pigs. Many of the above lesions were observed in monkeys. In addition, cutaneous lesions of the eyelids, face, ear canal and toe nails were also apparent. Except for the mouse, the lesions were comparable to those produced by dioxins in the same species of animal. This project is to be continued.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Halogenated dibenzofurans may occur in association with useful chlorinated cyclic compounds. In addition, there is evidence that they were contaminants of polychlorinated biphenyls and may have contributed in the Yusho accident. The pattern of toxicity associated with most of the isomers is not known. These facts must be known if assessment of the environmental health implication of the materials which contain these compounds is to be made.

PUBLICATIONS

Moore, J.A., McConnell, E.E., Dalgard, D.W., and Harris, M.W. Comparative Toxicity of Three Halogenated Dibenzofurans in Guinea Pigs, Mice and Rhesus Monkeys. Proceed. New York Academy of Science, New York, June, 1976, (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30040-04 EBCB												
PERIOD COVERED October 1, 1977 to September 30, 1978														
TITLE OF PROJECT (80 characters or less) Dose Response of 2,3,7,8-tetrachlorodibenzofuran on Drug Metabolizing Enzymes in the Rat														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT														
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI:</td> <td style="width: 33%;">J.A. Goldstein</td> <td style="width: 33%;">Pharmacologist</td> <td style="width: 33%;">EBCB NIEHS</td> </tr> <tr> <td>OTHER:</td> <td>P. Linko</td> <td>Biological Laboratory Technician</td> <td>EBCB NIEHS</td> </tr> <tr> <td></td> <td>R. Hass</td> <td>Senior Staff Fellow</td> <td>EBCB NIEHS</td> </tr> </table>			PI:	J.A. Goldstein	Pharmacologist	EBCB NIEHS	OTHER:	P. Linko	Biological Laboratory Technician	EBCB NIEHS		R. Hass	Senior Staff Fellow	EBCB NIEHS
PI:	J.A. Goldstein	Pharmacologist	EBCB NIEHS											
OTHER:	P. Linko	Biological Laboratory Technician	EBCB NIEHS											
	R. Hass	Senior Staff Fellow	EBCB NIEHS											
COOPERATING UNITS (if any) None														
LAB/BRANCH Environmental Biology and Chemistry Branch														
SECTION														
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709														
TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.1	OTHER: 0.1												
CHECK APPROPRIATE BOX(ES)														
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER														
☐ (a1) MINORS ☐ (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords) To determine whether the mixed effects of commercial 2,3,5,2',4',5'-hexachlorobiphenyl (HCB) on <u>drug metabolizing enzymes</u> can be explained by contamination by 44 ppm <u>2,3,7,8-tetrachlorodibenzofuran(TCDF)</u>.														

PROJECT DESCRIPTION

METHODS EMPLOYED: Female CD rats were given 3 daily i.p. doses of TCDF (0.1, 0.5, 2.5, 12.5, 63 and 313 $\mu\text{g/kg}$) and a spectrum of drug-metabolizing enzyme measured. For comparison, rats were dosed with 50 mg/kg of pure HCB, 50 mg/kg of commercial HCB and pure HCB plus 44 ppm TCDF. This study is compared with previously determined dose-response curves for 2,4,5,2',4',5'-HCB.

MAJOR FINDINGS AND PROPOSED COURSE: The TCDF which contaminates the commercial HCB isomer is sufficient to account for the induction of cytochrome P-448 and associated enzymes. Pure 2,4,5-HCB is a phenobarbital type inducer. This study is completed.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Chlorinated dibenzofurans occur in association with polychlorinated biphenyl (PCB) mixtures and may account for some of their toxicity because their potency is at least 10^4 greater. This study shows that even 99% pure isomers may contain sufficient amounts of dibenzofurans to account for their effects. This study adds to a previous study of the structure activity relationships of PCB's.

PUBLICATIONS

Goldstein, J.A., Hass, J.R., Linko, P., and Harvan, D.J. 2,3,7,8-Tetrachlorodibenzofuran in a Commercially Available Polychlorinated Biphenyl Isomer Identified as the Inducer of Hepatic Cytochrome P-448 and Aryl Hydrocarbon Hydroxylase in the Rat. Drug Metab. Dispos. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER 201 ES 30041-03 EBCB																				
PERIOD COVERED October 1, 1977 to September 30, 1978																						
TITLE OF PROJECT (80 characters or less) Immunochemistry of Hydrophobic Haptens																						
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																						
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 40%;">P. W. Albro</td> <td style="width: 35%;">Research Chemist</td> <td style="width: 10%;">EBCB NIEHS</td> </tr> <tr> <td>OTHER:</td> <td>M. I. Luster</td> <td>Staff Fellow</td> <td>EBCB NIEHS</td> </tr> <tr> <td></td> <td>K. Chae</td> <td>Staff Fellow</td> <td>EBCB NIEHS</td> </tr> <tr> <td></td> <td>A. Chaudhary</td> <td>Visiting Fellow</td> <td>EBCB NIEHS</td> </tr> <tr> <td></td> <td>J. D. McKinney</td> <td>Supervisory Research Chemist</td> <td>EBCB NIEHS</td> </tr> </table>			PI:	P. W. Albro	Research Chemist	EBCB NIEHS	OTHER:	M. I. Luster	Staff Fellow	EBCB NIEHS		K. Chae	Staff Fellow	EBCB NIEHS		A. Chaudhary	Visiting Fellow	EBCB NIEHS		J. D. McKinney	Supervisory Research Chemist	EBCB NIEHS
PI:	P. W. Albro	Research Chemist	EBCB NIEHS																			
OTHER:	M. I. Luster	Staff Fellow	EBCB NIEHS																			
	K. Chae	Staff Fellow	EBCB NIEHS																			
	A. Chaudhary	Visiting Fellow	EBCB NIEHS																			
	J. D. McKinney	Supervisory Research Chemist	EBCB NIEHS																			
COOPERATING UNITS (if any) Animal Husbandry Section, EBCB																						
LAB/BRANCH Environmental Biology and Chemistry Branch																						
SECTION Chemistry Section																						
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																						
TOTAL MANYEARS: 4.2	PROFESSIONAL: 2.5	OTHER: 1.7																				
CHECK APPROPRIATE BOX(ES)																						
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER																						
☐ (a1) MINORS ☐ (a2) INTERVIEWS																						
SUMMARY OF WORK (200 words or less - underline keywords)																						
A <u>radioimmunoassay</u> for chlorinated dibenzodioxins has been developed. One version of the assay responds to between 20 and 200 pg of dioxin, but is subject to interference. Another version responds to between 90 pg and 10 ng of dioxin. An adequate assay for <u>mono-2-ethylhexyl phthalate</u> in plasma has been completed. Several antisera to specific PCB isomers have been raised and are being characterized. Attempts are being made to raise an antiserum specific for <u>dibenzofurans</u>. Techniques for the presentation of these water-insoluble substances to the necessarily aqueous antibodies are being refined.																						

PROJECT DESCRIPTION

METHODS EMPLOYED: Synthetic techniques: condensation reactions, amination, reduction, mixed ester formation, active ester formation, convergent synthesis, spectroscopic and chromatographic characterization. Conjugation techniques: diazo coupling, mixed anhydride acylation, active ester acylation. Characterization of conjugates by UV spectroscopy, gel filtration, amino acid analysis, NMR spectrometry, and chemical assay of functional groups. Antibody production methods: standard procedures, with adjuvant, in rabbits. Antibody assay methods: double immunodiffusion, fluorescent antigen, radioimmunoassay.

MAJOR FINDINGS AND PROPOSED COURSE: An MEHP-RIA has been developed for MEHP in unextracted plasma. The sensitivity and specificity for MEHP has been confirmed by gas chromatographic procedures. The lower limit of sensitivity is 1 ng.

PCB - Antisera to 3,4,3',4'-PCB and 2,6,2',6'-PCB have been extensively characterized for sensitivity and specificity. These antisera react only with the specific isomer used for immunization and not with a variety of other PCBs, dioxin or furan. We are presently attempting to analyze various Arochlors and extracts of Yusho rice oil for PCB content. Results will be confirmed by gas chromatographic procedures. The limit of sensitivity for any of these particular PCBs is ~1 ng.

TCDD - Antisera with high sensitivity to 2,3,7,8-TCDD cross-react to various but lesser degrees with other chlorinated dioxins and some furans. This was expected since high sensitivity usually reflects decreased specificity. From standard curves employing pure TCDD and ¹²⁵I-iodovaleramide-2,3,7-TricDD, the lower level of sensitivity for TCDD is ~20-50 picograms. When clean-up extracts from spiked and non-spiked liver samples were tested, a large degree of interference occurred. We are modifying extraction procedures in an attempt to limit the degree of interference. Meanwhile, fat extracts appear more promising since little interference occurs when these samples are analyzed.

Synthesis - The following haptens have been synthesized for use in immunoassay development: 2-amino-4,5,3',4'-tetrachlorobiphenyl; 2-amino-3',4,4'-trichlorobiphenyl ether; 3-amino-2,6,2',6'-tetrachlorobiphenyl; 2-amino-4-chloro-3',4'-dimethylbiphenyl ether; 3,4,3',4'-tetramethylbiphenyl; 2,4,5,4'-tetrachlorobiphenyl ether; 3,4,4'-trichlorobiphenyl ether; 1-(5-¹²⁵I-iodovaleramide)-3,7,8-trichlorodibenzo-p-dioxin (also unlabeled); 2-(5-¹²⁵I-iodovaleramide)-4,5,3',4'-tetrachlorobiphenyl (also unlabeled); 3-(5-¹²⁵I-iodovaleramide)-2,6,2',6'-tetrachlorobiphenyl (also unlabeled); 4-amino-mono-(2-ethylhexyl) phthalate.

Current work includes increasing the number of compounds used to define antisera, and more particularly, attempting to improve the solubilization of these hydrophobes in assay media.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Immunoassay offers advantages of extreme specificity and sensitivity such that detection of hazardous compounds, mutated proteins, residues, etc. at biologically meaningful levels may be feasible with this approach. Most hapten-protein conjugates synthesized in the past have not been well characterized or reproducible, weaknesses hopefully to be overcome in the present project. Chlorinated diphenyl ether derivatives (dibenzodioxins and dibenzofurans) are currently being emphasized since some are exquisitely toxic and may be widespread environmental contaminants.

PUBLICATIONS

Luster, M.I., Albro, P.W., Chae, K., and Clark, G.: Radioimmunoassay for MEHP in unextracted plasma and serum. Clin. Chem. (in press).

Albro, P.W., Chae, K., Luster, M., McKinney, J.D., Chaudhary, S., Clark, G., Fawkes, J., and Corbett, J.: Radioimmunoassay for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Environ. Health Perspect. 20: 247, 1977. (abstract)

Luster, M.I., Albro, P.W., Faith, R.E., and Lawson, L.D.: Inability of passive antibodies to reverse the effects of dioxin toxicity. Chemosphere (in press).

Chae, K., Cho, L.K., and McKinney, J.D.: Synthesis of 1-amino-3,7,8-trichlorodibenzo-*p*-dioxin and 1-amino-2,3,7,8-tetrachlorodibenzo-*p*-dioxin as haptenic compounds. J. Agric. Food Chem. 25: 1207-1209, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30042-03 EBCB								
PERIOD COVERED October 1, 1977 to September 30, 1978										
TITLE OF PROJECT (80 characters or less) Effects of Environmental Contaminants on Cardiac Function										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT										
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI:</td> <td style="width: 33%;">E. W. Van Stee</td> <td style="width: 33%;">Physiologist</td> <td style="width: 33%;">EBCB NIEHS</td> </tr> <tr> <td></td> <td>M.P. Moorman</td> <td>Biomedical Engineer</td> <td>EBCB NIEHS</td> </tr> </table>			PI:	E. W. Van Stee	Physiologist	EBCB NIEHS		M.P. Moorman	Biomedical Engineer	EBCB NIEHS
PI:	E. W. Van Stee	Physiologist	EBCB NIEHS							
	M.P. Moorman	Biomedical Engineer	EBCB NIEHS							
COOPERATING UNITS (if any) Pathology Unit, EBCB										
LAB/BRANCH Environmental Biology and Chemistry Branch										
SECTION Inhalation Toxicology Section										
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709										
TOTAL MANYEARS: 0.40	PROFESSIONAL: 0.25	OTHER: 0.15								
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS										
SUMMARY OF WORK (200 words or less - underline keywords) This project is directed toward the detection of <u>functional changes</u> in the <u>cardiovascular system</u> associated with compounds of environmental significance to which <u>toxicologically significant exposure</u> would be expected by <u>inhalation</u>. The <u>cardiovascular effects</u> of brief inhalation exposures of rabbits and guinea pigs to 100 ppm of <u>morpholine</u> of the IV injection of micromolar quantities of the liquid in <u>aqueous solution</u> were unremarkable. The mechanism of a transitory <u>hypotension</u> associated with the presumed activation of vascular <u>muscarinic receptors</u> remained incompletely described.										

PROJECT DESCRIPTION

METHODS EMPLOYED: The effects of environmental chemicals on the heart are studied using isolated, perfused (Langedorff) rabbit hearts, anesthetized, open-chested guinea pigs, and isolated heart muscle preparations.

MAJOR FINDINGS AND PROPOSED COURSE: Exposure of guinea pigs anesthetized with ketamine with or without halothane to 100 ppm of morpholine for 15 minutes did not affect myocardial contractility or ventricular action potential configuration.

Closed-chested guinea pigs were anesthetized with ketamine and a carotid artery and jugular vein catheterized with PE 90 tubing. Blood pressure was recorded through the artery and infusions made through the vein. The IV injection of 4, 20, and 40 micromoles of morpholine in aqueous solution with the pH adjusted with HCl to 7.4 resulted in a dose dependent decrease in mean arterial blood pressure with an onset within seconds of the injection and recovery not exceeding one minute. The hypotensive effect of morpholine was not affected by ganglionic blockade with hexamethonium or adrenergic blockade with either phenoxybenzamine or propranolol. The response was unaffected by histamine H_1 and H_2 blockade. The effect was abolished by atropine, but unaffected by vagal section or pancuronium. Cardiodynamic changes were unremarkable and the effect was attributed to a direct effect on the vasculature involving muscarinic receptors.

The hypotensive effect was unaffected by pretreated with neostigmine and so the effect could not be explained on the basis of cholinesterase inhibition. Depletion of cholinergic presynaptic terminals with HC-3 which inhibits the reuptake of choline necessary for Ach resynthesis suggesting that the effect was probably not mediated by the presynaptic enhancement of Ach liberation. The conclusion remained that vascular muscarinic receptors were involved, perhaps by direct interaction with the morpholine.

Cylindrical segments of rabbit or guinea pig posterior vena cava were tested for morpholine-induced relaxation. At all reasonable preloads no relaxation was evident. At high concentrations morpholine caused a small contraction of venous smooth muscle. Similarly, guinea pigs ileal strips were not reproducibly responsive to morpholine, nor was luminal balloon pressure in the guinea pig cecum.

Morpholine is suspected to interact with muscarinic vascular receptors, presumably in the venous side of the circulation, but at unidentified anatomical locations.

A distillation of results obtained from a wide variety of experiments with morpholine in our laboratories does not suggest that there is sufficient justification for pursuing its investigation in much more detail based on a supposed environmental hazard.

The basic capability is retained within the laboratory to evaluate other potential toxicants of environmental significance.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Based on the experiments described herein we would expect no acute effects of the exposure by inhalation for 15 min to 100 ppm or the injection of micromolar quantities of the liquid in solution in the cardiovascular systems of guinea pigs or rabbits exposed to morpholine.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30044-02 EBCB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Toxicology of Environmental Chemicals		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: E. W. Van Stee E. G. Tombropoulos H. F. Lam OTHER: M. P. Moorman A. G. E. Wilson F. Harrington P. Wynns	Physiologist Research Chemist Visiting Fellow Biomedical Engineer Visiting Associate Biological Laboratory Technician Biological Aid	EBCB NIEHS EBCB NIEHS EBCB NIEHS EBCB NIEHS LP NIEHS EBCB NIEHS EBCB NIEHS
COOPERATING UNITS (if any) Pharmacokinetics Section, LP, NIEHS		
LAB/BRANCH Environmental Biology and Chemistry Branch		
SECTION Inhalation Toxicology Section		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.2	OTHER: 0.2
CHECK APPROPRIATE BOX(ES)		
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER		
☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) <u>Morpholine</u> has been selected as the first chemical for evaluation. Morpholine is a cheap and widely used solvent. It is a heterocyclic secondary <u>amine</u> that would be expected to be excreted in the urine by way of the tubular organic base excretory mechanism. One of the apparent reasons why this compound has not been studied in the past is the difficulty of the analysis in biological fluids. A considerable amount of time has been devoted to establishing this methodology and we now have a reliable gas chromatographic method for its determination in blood and urine. A rabbit nose exposure unit has been assembled to include automatic GC monitoring. The pharmacokinetics and renal handling of morpholine are being studied.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Rabbits, rats and mice are exposed to gases, e.g. morpholine vapor, in dynamic flow-through exposure chambers of the Hinners-Punte design. Median lethal concentration (LC₅₀) and histopathological damage are determined by the usual methods. Tissue levels of compounds are determined by gas chromatographic methods. Renal studies *in vivo* are conducted using clearance technics and *in vitro* using slice-to-medium ratios, slice uptake, and slice washout methods.

MAJOR FINDINGS AND PROPOSED COURSE: A re-evaluation of the toxicity of morpholine shows that this compound has a higher inhalation toxicity than was previously suspected(1). Acute toxicity studies gave the LC₅₀ values in male and female rats to be 2250 and 2150 ppm respectively, and male and female mice to be 1450 and 1900 ppm respectively. In a subacute study, rats were exposed to 450 ppm morpholine, 6hr/day, 5 days/week for 8 weeks. Morpholine was found to be an anorectic and has an effect on the absorption and utilization of the food consumed. It caused histological changes in the lungs, liver, kidneys and heart. Studies are in progress to determine: (a) the hematological effects of morpholine (blood gases, blood types); (b) the ultrastructural change in the affected organs.

In vivo studies of the distribution of inhaled morpholine throughout the body revealed that concentrations achieved in the kidney were highest of those achieved in any organ. This was not unexpected *a priori* since the amine would be expected to be excreted by the kidney via the tubular organic base transport system. Characterization of this tubular transport system *in vitro* has begun and kidney slices have been demonstrated to take up morpholine from the medium, probably in competition with, and via the same mechanism as, tetraethylammonium. Prior exposure of rats to morpholine results in enhanced PAH uptake *in vitro*, a non-specific sign of renal toxicity exhibited by such diverse other nephrotoxins as cadmium and gentamicin.

Two pairs of rabbits were exposed to morpholine, 250 ppm, for 7 hr. One pair was used to take blood samples during intervals throughout the exposure and then killed at the end. Tissue concentrations of morpholine were performed at this time. The second pair were not sampled until one hour after the end of the exposure at intervals thereafter for 24 hr. They were killed at that time and the tissue concentrations determined.

Values for the determinations are expressed as means for the pair.
First pair (sampled during exposure); killed after 7 hr exposure:

<u>Duration of exposures, hr</u>	<u>ug/g tissue or ml blood, urine</u>
(Blood)	
2	12.8
3	33.25
4	37.61
5	57.07
6	77.58
7	85.94
(Urine)	
7	194.91
Liver	45
Lung	57.9
Kidney	100.4
Brain	70.4

Second pair (exposed 7 hr, killed 24 hr later:

<u>Postexposure time, hr</u>	<u>ug/g tissue or ml blood, urine, bile</u>
(Blood)	
1	75.83
2	52.49
3, 2 1/2	38.72
24	20.3
Bile	156.63
Urine	trace
Liver	trace
Lungs	trace
Kidney	10.72
Brain	trace
Muscle	trace
Fat	not detectable
Spleen	30.57
Testicles	trace
Adrenals	66.45

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

The pulmonary and cardiovascular systems are tightly integrated with renal function. The assessment of the impact on the organism of exposure to environmental contaminants is dependent on a comprehensive understanding of the effects of impairment of interactive physiological functions. The role of the kidneys as regulators of cardiovascular function requires the scientific investigation of chemicals for which the target organ may be the kidney.

When indicated and practicable, our general approach to studies of the inhalation toxicology of gases will include closely integrated studies of pulmonary, cardiovascular, and renal function.

PUBLICATIONS

Lam, H.F. and Van Stee, E.W.: A re-evaluation of the toxicity of morpholine. Fed. Proc. 37: 679, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30045-02 EBCB																
PERIOD COVERED October 1, 1977 to September 30, 1978																		
TITLE OF PROJECT (80 characters or less) Development of an Automatic Small Animal Inhalation Facility																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																		
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">M. P. Moorman</td> <td style="width: 35%;">Biomedical Engineer</td> <td style="width: 15%;">EBCB NIEHS</td> </tr> <tr> <td></td> <td>E. W. Van Stee</td> <td>Physiologist</td> <td>EBCB NIEHS</td> </tr> <tr> <td>OTHER:</td> <td>R. Dennis</td> <td>Machinist</td> <td>RSB NIEHS</td> </tr> <tr> <td></td> <td>F. Harrington</td> <td>Biological Laboratory Technician</td> <td>EBCB NIEHS</td> </tr> </table>			PI:	M. P. Moorman	Biomedical Engineer	EBCB NIEHS		E. W. Van Stee	Physiologist	EBCB NIEHS	OTHER:	R. Dennis	Machinist	RSB NIEHS		F. Harrington	Biological Laboratory Technician	EBCB NIEHS
PI:	M. P. Moorman	Biomedical Engineer	EBCB NIEHS															
	E. W. Van Stee	Physiologist	EBCB NIEHS															
OTHER:	R. Dennis	Machinist	RSB NIEHS															
	F. Harrington	Biological Laboratory Technician	EBCB NIEHS															
COOPERATING UNITS (if any) None																		
LAB/BRANCH Environmental Biology and Chemistry Branch																		
SECTION Inhalation Toxicology Section																		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																		
TOTAL MANYEARS: 1/3	PROFESSIONAL: .6	OTHER: .7																
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																		
SUMMARY OF WORK (200 words or less - underline keywords) This project involves the design and implementation of an <u>automatic data processing</u> system to monitor and control gas concentrations in a <u>small animal inhalation exposure facility</u> . Included in this effort are the development of hardware, software and operational procedures necessary for the complete utilization of the system.																		

PROJECT DESCRIPTION

METHODS EMPLOYED: Data acquisition and feedback control theory have been used to develop an electronic calculator based sampled data control system capable of regulating 9 chambers on a time multiplex basis.

MAJOR FINDINGS AND PROPOSED COURSE: Since this system must regulate gases generated from compounds with different physical properties, it has been necessary to design a control system capable of measuring certain characteristics of each generating system and adapting the control equations to optimize responses for each particular compound.

Because equipment calibration is a significant factor in system performance statistical procedures have been implemented to evaluate daily calibrations and long term system performance.

In order to better define the exposure environment, temperature and humidity are monitored and animal excrement actively removed during the course of the exposure.

Special purpose procedures and software necessary to handle unusual exposures and circumstances must be developed to bring this system to its full potential.

The realization of maximum usefulness of this project requires detailed documentation of system hardware, software, and operating procedures. System performance must be documented through use.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

1. Machine control of inhalation chambers can produce an accurately controlled and documented exposure resulting in reduced technical error.
2. Statistical evaluation of periodic calibrations can quantify monitoring accuracy and identify many calibration errors.
3. Documentation of the exposure conditions and profiles can be summarized in a compact and meaningful format eliminating the need for hand analysis of chamber output data.
4. More complicated exposures such as time varying concentrations of multiple compounds are possible with machine control more than human operators.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30050-02 EBCB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Chemical Conjugation of Specific ¹³ C-Labeled Benzo[a]pyrene Oxides to Glutathione: Regiospecificity and Stereospecificity		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: OTHER:	J. D. McKinney R. H. Cox O. Hernandez B. Smith J. Bend	Supervisory Research Chemist Research Chemist Visiting Associate Staff Fellow Visiting Scientist EBCB NIEHS EBCB NIEHS EBCB NIEHS LP NIEHS LP NIEHS
COOPERATING UNITS (if any) Marine Pharmacology Section, LP, NIEHS, and Midwest Research Institute (Procurement Contract with NCI, No. N01-CP-33387. Supplemented by NIEHS).		
LAB/BRANCH Environmental Biology and Chemistry Branch		
SECTION Chemistry Section		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.8	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Nuclear magnetic resonance (<u>NMR</u>) <u>spectroscopy</u> is being used to investigate the chemical <u>conjugation</u> of specific ¹³ C-labeled <u>benzo[a]pyrene</u> oxides and other arene oxides to <u>glutathione</u> , enzymatically and non-enzymatically. The <u>regiospecificity</u> and <u>stereospecificity</u> of the reaction will be determined. Mechanistic elaboration of the binding process is important in understanding, preventing, and predicting the mechanism(s) of action of such compounds. Several specifically ¹³ C-labeled benzo[a]pyrene derivatives have been obtained and studies of their chemical and biological conjugation have been initiated.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Fourier transform ^{13}C and ^1H nuclear magnetic resonance (NMR) spectroscopy; organic syntheses, in vitro biological experiments.

MAJOR FINDINGS AND PROPOSED COURSE: Several benzo[a]pyrene derivatives of high purity and definition including two oxides, one dihydrodiol and one diol oxide, labeled at specific positions and known enrichments with non-radioactive ^{13}C have been obtained. Studies with benzo[a]pyrene-4,5-oxide-4,5- $^{13}\text{C}_2$ have been initiated to determine the preferred position of attack on the arene oxide by glutathione and the stereospecificity of the addition product. The detoxifying glutathione S-transferase enzyme system previously shown to covalently bind certain epoxides will be used. Studies with styrene oxide have shown that both positional isomers (attachment of sulfur of glutathione to the epoxide) are produced in approximately equal amounts, with little difference in the isomer ratio between the enzymatic and non-enzymatic reactions. High-pressure liquid chromatographic (HPLC) techniques have been developed to separate the isomers produced. Future studies should include competition experiments to determine which biological nucleophiles are most reactive and the enzymatic versus non-enzymatic nature of the ring opening process and, if possible, elucidation of the required microenvironment for the covalent binding of such arene oxides. ^{13}C -NMR spectra of the various oxygenated derivatives will be recorded routinely for reference purposes.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Certain arene oxides are known to bind covalently to nucleic acids, and they have been implicated as carcinogens and mutagens which can be derived from the parent compounds. The abundant detoxifying glutathione S-transferase enzyme system is also likely to covalently bind these oxides. Such mechanistic elaboration of the process will aid in understanding, preventing, and predicting the mechanism(s) of action of these compounds.

PUBLICATIONS

Hernandez, O., Cox, R.H., Smith, B., Bend, J.R., and McKinney, J.D.: Structural properties and synthesis of glutathione conjugates of oxides of styrene and benzo[a]pyrene. Symposium on Polynuclear Aromatic Hydrocarbons, Columbus, Ohio, October, 1978. (abstract)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30051-02 EBCB																								
PERIOD COVERED October 1, 1977 to September 30, 1978																										
TITLE OF PROJECT (80 characters or less) Characterization of Specific Binding Modes of Organics and Inorganics: The Toxic Polyhalogenated Aromatic Hydrocarbons																										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">J.D. McKinney</td> <td style="width: 35%;">Supervisory Research Chemist</td> <td style="width: 15%;">EBCB NIEHS</td> </tr> <tr> <td>OTHER:</td> <td>P. Singh</td> <td>Research Chemist</td> <td>EBCB NIEHS</td> </tr> <tr> <td></td> <td>R.H. Cox</td> <td>Research Chemist</td> <td>EBCB NIEHS</td> </tr> <tr> <td></td> <td>P.A. Albro</td> <td>Research Chemist</td> <td>EBCB NIEHS</td> </tr> <tr> <td></td> <td>E.E. McConnell</td> <td>Veterinary Pathologist</td> <td>EBCB NIEHS</td> </tr> <tr> <td></td> <td>J.A. Goldstein</td> <td>Pharmacologist</td> <td>EBCB NIEHS</td> </tr> </table>			PI:	J.D. McKinney	Supervisory Research Chemist	EBCB NIEHS	OTHER:	P. Singh	Research Chemist	EBCB NIEHS		R.H. Cox	Research Chemist	EBCB NIEHS		P.A. Albro	Research Chemist	EBCB NIEHS		E.E. McConnell	Veterinary Pathologist	EBCB NIEHS		J.A. Goldstein	Pharmacologist	EBCB NIEHS
PI:	J.D. McKinney	Supervisory Research Chemist	EBCB NIEHS																							
OTHER:	P. Singh	Research Chemist	EBCB NIEHS																							
	R.H. Cox	Research Chemist	EBCB NIEHS																							
	P.A. Albro	Research Chemist	EBCB NIEHS																							
	E.E. McConnell	Veterinary Pathologist	EBCB NIEHS																							
	J.A. Goldstein	Pharmacologist	EBCB NIEHS																							
COOPERATING UNITS (if any) Comparative Biology Section																										
LAB/BRANCH Environmental Biology and Chemistry Branch																										
SECTION Chemistry Section																										
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																										
TOTAL MANYEARS: 1.4	PROFESSIONAL: 1.2	OTHER: 0.2																								
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																										
SUMMARY OF WORK (200 words or less - underline keywords) The exact molecular structures of some highly toxic polyhalogenated aromatic hydrocarbons have been obtained by X-ray crystallographic measurements or estimated based on postulated structures. The requirements of molecular symmetry and size as determined by the number, kind, and positions of halogens, planarity, interatomic distances and overall stereoelectronics suggest that a specific biological receptor may be involved which could account for the common toxic pattern.																										

POSITION DESCRIPTION

METHODS EMPLOYED: X-ray crystallography with associated equipment and techniques was used primarily in this phase of the work. Variable temperature high resolution multi nuclei nuclear magnetic resonance (NMR) spectroscopy using specifically labeled (^{13}C , ^{19}F , ^1H , etc.) compounds where possible for studying complex molecular interactions is also used when solubility is not a problem. Isolation and characterization of specific binding site(s) in body tissue and fluid using standard biochemical methods.

MAJOR FINDINGS AND PROPOSED COURSE: Early attempts to study specific binding modes of the toxic polyhalogenated aromatic hydrocarbons by NMR was complicated by solubility problems. The molecular structures of some of these compounds have now been examined using X-ray crystallography. The molecular structure of dibenzo-p-dioxin, the parent nucleus of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), published in the literature seemed to us to be in error. This structure was reinvestigated which yielded a much improved molecular geometry for the dioxin molecule. In another structural study some important distances between halogen atoms in TCDD, 2,3,7,8-tetrachlorodibenzofuran (TCDF), 3,3',4,4',5,5'-hexachlorobiphenyl, and 2,3,6,7-tetrachloro- and tetrabromonaphthalenes were estimated based on postulated structures, except for TCDD, whose X-ray structure is known. The structure of one polychlorinated biphenyl compound, viz. 2,2',4,4',6,6'-hexachlorobiphenyl has been completed, which shows that the two benzene rings are almost perpendicular to each other (the angle of twist being 87.3°) and, therefore, exhibits the maximum ortho effect. The structurally similar compounds as determined by these techniques also afford a common toxic pattern in the guinea pig. The requirements of molecular symmetry and size suggest that a specific biological receptor may be involved. Future studies will examine the X-ray structures of products from cocrystallization of the toxic compounds with model biological substrates.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

There is increasing evidence that certain highly toxic halogenated hydrocarbons may have specific binding receptors in biological systems which differ quantitatively in their ability to bind both halogenated and non-halogenated planar molecules. Binding propensity and toxicity may be correlatable. An understanding of the specific molecular level interactions involved in binding may permit one to predict, prevent, or reverse them.

PUBLICATIONS

Singh, P., and McKinney, J.D.: Dibenzo-p-dioxin: A refinement. Acta Crystallographica (in press).

McConnell, E.E., and McKinney, J.D.: Exquisite toxicity in the guinea pig to structurally similar halogenated dioxins, furans, biphenyls and naphthalenes. Society of Toxicology Meeting, San Francisco, March, 1978. (abstract)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30052-02 EBCB												
PERIOD COVERED October 1, 1977 to September 30, 1978														
TITLE OF PROJECT (80 characters or less) ¹³ C-Nuclear Magnetic Resonance Study of Chlorinated Polycyclodiene Pesticides														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 30%;">J. D. McKinney</td> <td style="width: 30%;">Supervisory Research Chemist</td> <td style="width: 25%;">EBCB NIEHS</td> </tr> <tr> <td></td> <td>R. H. Cox</td> <td>Research Chemist</td> <td>EBCB NIEHS</td> </tr> <tr> <td>OTHER:</td> <td colspan="3">None</td> </tr> </table>			PI:	J. D. McKinney	Supervisory Research Chemist	EBCB NIEHS		R. H. Cox	Research Chemist	EBCB NIEHS	OTHER:	None		
PI:	J. D. McKinney	Supervisory Research Chemist	EBCB NIEHS											
	R. H. Cox	Research Chemist	EBCB NIEHS											
OTHER:	None													
COOPERATING UNITS (if any) None														
LAB/BRANCH Environmental Biology and Chemistry Branch														
SECTION Chemistry Section														
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709														
TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0												
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords) ¹³ C-Nuclear Magnetic Resonance (NMR) is being used to characterize several chlorinated polycyclodiene pesticides and some of their phototransformation and potential metabolic products. ¹³ C-NMR was shown to be superior to proton NMR for identification of these compounds and elucidation of their peculiar <u>electronic interactions</u> which underlie their <u>biological activity</u> .														

PROJECT DESCRIPTION

METHODS EMPLOYED: Fourier transform ^{13}C nuclear magnetic resonance (NMR) spectroscopy and shift and relaxation reagents.

MAJOR FINDINGS AND PROPOSED COURSE: The ^{13}C NMR spectra of several chlorinated polycyclodiene pesticides including aldrin, dieldrin, endrin, and heptachlor, and twenty three of their phototransformation and potential metabolic products (derived from synthesis), have been obtained and interpreted.

^{13}C -NMR was shown to be superior to proton NMR for the identification of these compounds and will aid in elucidating the peculiar electronic interactions in these polycyclic systems which underlie their biological activity. This study was made as comprehensive as possible within the limitations of our synthetic capabilities.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The ultimate fate of the chlorinated polycyclodiene pesticides in the environment continues to be investigated. Therefore, more definitive techniques to identify their various possible transformation products are needed. Furthermore, it is likely that the biological activity associated with many of these compounds is a function of their peculiar electronic interactions, intra- and intermolecularly.

PUBLICATIONS

Cox, R.H. and McKinney, J.D.: Carbon-13 NMR studies of some chlorinated polycyclodiene pesticides. Org. Mag. Res. (in press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30055-03 EBCB												
PERIOD COVERED October 1, 1977 to September 30, 1978														
TITLE OF PROJECT (80 characters or less) Mechanism of 2,3,7,8-Tetrachlorodibenzo-p-dioxin Toxicity in Rats														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0"> <tr> <td>PI:</td> <td>E.E. McConnell</td> <td>Veterinary Pathologist</td> <td>EBCB NIEHS</td> </tr> <tr> <td>OTHER:</td> <td>P. W. Albro</td> <td>Research Chemist</td> <td>EBCB NIEHS</td> </tr> <tr> <td></td> <td>H. B. Matthews</td> <td>Research Chemist</td> <td>LP NIEHS</td> </tr> </table>			PI:	E.E. McConnell	Veterinary Pathologist	EBCB NIEHS	OTHER:	P. W. Albro	Research Chemist	EBCB NIEHS		H. B. Matthews	Research Chemist	LP NIEHS
PI:	E.E. McConnell	Veterinary Pathologist	EBCB NIEHS											
OTHER:	P. W. Albro	Research Chemist	EBCB NIEHS											
	H. B. Matthews	Research Chemist	LP NIEHS											
COOPERATING UNITS (if any) None														
LAB/BRANCH Environmental Biology and Chemistry Branch														
SECTION Comparative Biology Section														
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709														
TOTAL MANYEARS: 0.3	PROFESSIONAL: 0.2	OTHER: 0.1												
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this study is to investigate the <u>mechanism of toxicity</u> and <u>cause of death</u> in <u>rats</u> exposed to 2,3,7,8-tetrachlorodibenzo-p- dioxin. Specifically, the affect of <u>TCDD</u> on <u>fat absorption</u> , <u>metabolism</u> and <u>transport</u> from the small intestine.														

PROJECT DESCRIPTION

METHODS EMPLOYED: Fischer 344 rats were given a single oral LD₁₀₀ (80 µg/kg) of TCDD in corn oil. At various times thereafter, they were given 1 ml of olive oil. At 2, 4 and 6 hr after olive oil, portions of small intestine were taken for light and electron microscopy. Subsequent quantitative studies used C¹⁴ labeled triolin and C¹⁴ oleic acid.

MAJOR FINDINGS AND PROPOSED COURSE: As early as 3 days post TCDD exposure, there was a reduction in the rate of fat absorption from the small intestine. That absorbed remained in the intestinal epithelial cell longer than normal. In addition, ultrastructural analysis of the cell indicated that the fat globules are much larger than normal and are poorly transferred out of the cell. There was a reduction in the rate of absorption of both labeled compounds in TCDD treated compounds. In addition, TCDD causes a transient reduction in levels of pancreatic enzymes in the intestine. Project will be continued.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: TCDD is a highly toxic chemical that may be found as an unwanted contaminant of useful compounds. The lesions of toxicity have been described in several animal species, both in acute and chronic dosing regimens. However, in those cases where death occurs, it is difficult to explain the organopathologic basis. Most animals appear to waste away in a starvation-like manner. Halogenated biphenyl and dibenzofuran toxicity produces a similar syndrome. It would be extremely useful to be able to explain the mode of death, in the hope that preventative therapy could then be developed. It appears that TCDD interferes with the absorption and transport of lipids. If the absorption of other nutrients are similarly affected this may explain part of the disease syndrome.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30058-01 EBCB						
PERIOD COVERED October 1, 1977 to September 30, 1978								
TITLE OF PROJECT (80 characters or less) Synthesis of Thromboxanes and Thromboxane Analogs								
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%;"> <tr> <td style="width: 33%;">PI: O. Hernandez</td> <td style="width: 33%;">Visiting Associate</td> <td style="width: 33%;">EBCB NIEHS</td> </tr> <tr> <td>OTHER: S. Chaudhary</td> <td>Visiting Fellow</td> <td>EBCB NIEHS</td> </tr> </table>			PI: O. Hernandez	Visiting Associate	EBCB NIEHS	OTHER: S. Chaudhary	Visiting Fellow	EBCB NIEHS
PI: O. Hernandez	Visiting Associate	EBCB NIEHS						
OTHER: S. Chaudhary	Visiting Fellow	EBCB NIEHS						
COOPERATING UNITS (if any) None								
LAB/BRANCH Environmental Biology and Chemistry Branch								
SECTION Chemistry Section								
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709								
TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.9	OTHER: 0.0						
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS								
SUMMARY OF WORK (200 words or less - underline keywords) Thromboxanes are new kinds of metabolites derived from the prostaglandin endoperoxides <u>PGE₂</u> and <u>PGH₂</u> . Research seeking to understand the basis for the potent biological activity displayed by these compounds is limited by the lack of <u>availability</u> of samples. The purpose of this work is (1) to design a <u>practical synthesis</u> for the preparation of the title compounds and (2) to study the <u>chemical</u> and <u>biological properties</u> of thromboxanes.								

PROJECT DESCRIPTION

METHODS EMPLOYED: Selective functionalization of hydroxyl groups, Claisen rearrangement, high pressure liquid chromatography, chemical ionization mass spectrometry, proton magnetic resonance spectroscopy.

MAJOR FINDINGS AND PROPOSED COURSE: A chemical synthesis of thromboxane B_2 was completed. A unique feature of this work is the use of a carbohydrate² to generate optically pure intermediates as those found in the metabolic material. The synthetic scheme also provides a way of making these compounds in large quantities as required for extensive chemical and biological studies.

Thromboxane B_2 is a potential precursor for thromboxane A_2 , a possibility which will be explored.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Thromboxanes, and in particular thromboxane A_2 , are known to play a decisive role in several critical systems of the human² body. The mechanism by which this biological activity is exerted, remains unclear. A detailed study of the chemical and biological properties of thromboxanes is of great significance towards the understanding of the physiological role of thromboxanes.

PUBLICATIONS

Hernandez, O.: Chiral synthesis of thromboxane B_2 intermediates. Tetrahedron Lett. 219-222, 1978.

Hernandez, O., McKinney, J.D., Bend, J.R., Eling, T.E., and Anderson, M.W.: Chiral synthesis of thromboxane B_2 . Environ. Health Persp., 20: 245. 1977. (abstract)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30059-01 EBCB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Development of New Synthetic Procedures and Reactions.		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: O. Hernandez OTHER: S. Chaudhary R. Cox	Visiting Associate Visiting Fellow Research Chemist	EBCB NIEHS EBCB NIEHS EBCB NIEHS
COOPERATING UNITS (if any) None		
LAB/BRANCH Environmental Biology and Chemistry Branch		
SECTION Chemistry Section		
INSTITUTE AND LOCATION NIH, NIEHS, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.7	PROFESSIONAL: 0.7	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The ability to effect selective transformations in molecules containing chemically similar groups is of great value in synthetic organic chemistry since it expands the choice of starting materials and minimizes the use of separation procedures. The purpose of this work is to develop methods which allow specific derivatization of primary or secondary alcohols in molecules containing both functionalities. Expertise in synthetic organic chemistry is reflected in support and collaborative activities with other groups in the Institute.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Carbon magnetic resonance, proton magnetic resonance spectroscopy and the usual synthetic apparatus and equipment.

MAJOR FINDINGS AND PROPOSED COURSE: Procedures have been developed for the selective preparation of triphenylmethyl- and *t*-butyldimethylsilyl ethers of primary alcohols. These derivatives are frequently used in organic synthesis for the temporary masking of alcohols. The method is based on mechanistic considerations of the pyridine catalyzed alkylation of alcohols. Further work will establish if the same selectivity is achievable with other commonly used hydroxyl protective groups.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: An active research program on organic synthesis contributes to maintain a high level of expertise on this important discipline in the Chemistry Section. This expertise is reflected in support and collaborative activities with other groups in the Institute. As an example of this function, a chemical synthesis of thromboxane B₂ was based on the selective functionalization of the hydroxyl groups in glucose.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30060-01 EBCB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Structure of Fungal Metabolites		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: R.H. Cox OTHER: None Research Chemist EBCB NIEHS		
COOPERATING UNITS (if any) National Peanut Laboratory, Dawson, Georgia U.S.D.A., Georgia-South Carolina Area, Tifton, Georgia		
LAB/BRANCH Environmental Biology and Chemistry Branch		
SECTION Chemistry Section		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) <u>¹H- and ¹³C-Nuclear Magnetic Resonance (NMR)</u> is being used to characterize toxic fungal metabolites isolated from cereal crops. New structures deter- mined include 32-hydroxy-4 β ,8 α , 15-triacetoxy-12,13-epoxytrichothec-9-ene (neosolanol monoacetate) from <u>Fusarium tricinctum</u> isolated from peanuts, and <u>hydroxyterphenyllin</u> from <u>Aspergillus dandidus</u> found growing on unbleached flour.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Fourier transform ^1H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy.

MAJOR FINDINGS AND PROPOSED COURSE: Fusarium tricinctum was isolated from peanuts remaining in the soil after harvest. The mold produced a new toxic metabolite belonging to the Trichothecene family of mycotoxins. The toxin was shown by NMR and chemical techniques to be 3 α -hydroxy-4 β ,8 α ,15-triacetoxy-12,13-epoxytrichothec-9-ene and assigned the trivial name of neosolaniol monoacetate. The mean lethal dose of the toxin in 1-day-old cockerels was 0.789 mg/kg. The toxin was shown to be a potent plant growth inhibitor to 10^{-6}M in wheat coleoptile tests and possesses similar dermal toxicity to another trichothecene, T-2 toxin, in rabbit skin tests.

Two metabolites were isolated from Aspergillus candidus found growing on unleached flour. One of the metabolites was identified as terphenyllin (2',5'-dimethoxy-4,3',4'-tri-hydroxy-p-terphenyl), the other was a new metabolite, hydroxyterphenyllin (2',5'-dimethoxy-3,4,3',4'-tetrahydroxy-p-terphenyl).³ The former significantly inhibited wheat coleoptile growth only at 10^{-6}M ; the latter inhibited growth to 10^{-5}M .

Previous studies have shown terphenyllin to be active against HeLa cells. Testing of hydroxyterphenyllin for leukemia and HeLa cell activity is presently underway at NIH. If the biological activity of the terphenyllins is directly related to the placement and number of hydroxy groups on either, or all, of the three phenyl rings, then the basic template offers an interesting starting point for further production of medicinal chemicals of significant value.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Molds found growing on cereal crops in many instances produce toxic fungal metabolites that may possibly ultimately end up in the human food chain (i.e. the aflatoxins). Therefore, identification of these metabolites and screening for biological activity are needed. Furthermore, it is likely that some of the metabolites may possess biological activity beneficial to man.

PUBLICATIONS

Lansden, J.A., Cole, R.J., Dorner, J.W., Cox, R.H., Cutler, H.G. and Clark, J.P.: A new trichothecene mycotoxin isolated from Fusarium tricinctum. J. Agric. Food Chem. 26: 246-249, 1978.

Cutler, H.G., LeFiles, J.H., Crumley, F.G. and Cox, R.H.: Hydroxyterphenyllin: A novel fungal metabolite with plant growth regulating properties. J. Agric. Food Chem. (in press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30061-01 EBCB																								
PERIOD COVERED October 1, 1977 to September 30, 1978																										
TITLE OF PROJECT (80 characters or less) Chemical Characterization of Potentially Complex Toxicological Mixtures. Yusho Oil and Used Transformer Fluids.																										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																										
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">J. D. McKinney</td> <td style="width: 35%;">Supervisory Research Chemist</td> <td style="width: 15%;">EBCB NIEHS</td> </tr> <tr> <td>OTHER:</td> <td>M. Morita</td> <td>Guest Worker - Tokyo Metropolitan</td> <td>EBCB NIEHS</td> </tr> <tr> <td></td> <td></td> <td>Research Laboratory of Public Health</td> <td></td> </tr> <tr> <td></td> <td>P. A. Albro</td> <td>Research Chemist</td> <td>EBCB NIEHS</td> </tr> <tr> <td></td> <td>J. R. Hass</td> <td>Senior Staff Fellow</td> <td>EBCB NIEHS</td> </tr> <tr> <td></td> <td>E. E. McConnell</td> <td>Veterinary Pathologist</td> <td>EBCB NIEHS</td> </tr> </table>			PI:	J. D. McKinney	Supervisory Research Chemist	EBCB NIEHS	OTHER:	M. Morita	Guest Worker - Tokyo Metropolitan	EBCB NIEHS			Research Laboratory of Public Health			P. A. Albro	Research Chemist	EBCB NIEHS		J. R. Hass	Senior Staff Fellow	EBCB NIEHS		E. E. McConnell	Veterinary Pathologist	EBCB NIEHS
PI:	J. D. McKinney	Supervisory Research Chemist	EBCB NIEHS																							
OTHER:	M. Morita	Guest Worker - Tokyo Metropolitan	EBCB NIEHS																							
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	P. A. Albro	Research Chemist	EBCB NIEHS																							
	J. R. Hass	Senior Staff Fellow	EBCB NIEHS																							
	E. E. McConnell	Veterinary Pathologist	EBCB NIEHS																							
COOPERATING UNITS (if any) Comparative Biology Section																										
LAB/BRANCH Environmental Biology and Chemistry Branch																										
SECTION Chemistry Section																										
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																										
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.4	OTHER: 0.1																								
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																										
SUMMARY OF WORK (200 words or less - underline keywords) Rice oil contaminated with PCB-based heat exchange fluid in Japan (<u>Yusho oil</u>) has been analyzed primarily for content of <u>polyhalogenated aromatics</u> . This cursory examination has confirmed the presence of <u>transformation/rearrangement products</u> of PCB's among other products suggesting this would be a complex toxicological mixture which is not representative of PCB's. Similarly, preliminary examination of new and used <u>transformer fluids</u> from this country indicate that such PCB-based fluids do undergo considerable alterations and rearrangements during use. The complex mixtures generated would be expected to have <u>complex toxicological behavior</u> as well.																										

PROJECT DESCRIPTION

METHODS EMPLOYED: A variety of chromatographic and spectroscopic methods have been used to isolate and identify fractions/components of these complex mixtures including gas, gel permeation, high pressure liquid and other column chromatography and ultraviolet, infrared and nuclear magnetic resonance, and mass spectrometry. Neutron activation analysis was also used.

MAJOR FINDINGS AND PROPOSED COURSE: The Yusho oil examined was found to contain three major chlorinated hydrocarbon components. In addition to the expected polychlorinated biphenyls (PCB's), there were also dimers and trimers of biphenyl. The levels were estimated to be 200 µg/ml, 376 µg/ml and 58 µg/ml, respectively. Further characterization of each of these mixtures was done to a limited extent. Of the toxic biphenyls previously studied in our laboratories, only the 3,4,3',4'-tetrachlorobiphenyl was found at about 0.2 µg/ml in the contaminated rice oil. The presence of polychlorinated dibenzofurans at higher levels than found in the precursor Kaneclor 400 was confirmed. Preliminary neutron activation analysis on Yusho oil and its fractions remains to be explained. Toxicological studies (in guinea pigs) of Yusho oil and its fractions described here are given in another report.

Preliminary examinations of new and used PCB-based transformer fluids have been carried out. The new mixture appears to be a simple mixture of Aroclor 1254 and trichlorobenzene (1,2,4- and 1,2,3-isomers) with only a trace amount of tetra-isomers. The old fluid is an extremely complex mixture of mono to tetrachlorobenzenes, PCB's and other aromatics. The PCB mixture does not resemble any standard Aroclor. Neutron activation analysis indicated the presence of significant amounts of bromine (approx. 60 to 100 ppm) and a small amount of zinc (1 to 2 ppm). Tin was present at less than 5 ppm. Neither of the fluids contain mineral oil or silicone oil. Further work will continue to determine the presence of other possible transformation/rearrangement products in the used transformer fluid such as biphenyl dimers, trimers, and dibenzofurans, etc.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

PCBs are widely dispersed contaminants of the environment partly as a result of their industrial use as transformer and heat exchange fluids. In attempting to understand their environmental fate and distribution and associated toxicological impact, it is of considerable interest to determine the nature and extent of the alterations and rearrangements of such PCB-based fluids after extended use at high temperatures. Such thermodynamically derived PCB mixtures appear to present considerably different toxicological problems as evidenced by the "Yusho disease" of Japan.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30062-01 EBCB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Immunochemistry of Dioxin Action on the Lymphocyte		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: M. I. Luster OTHER: None Staff Fellow EBCB NIEHS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Environmental Biology and Chemistry Branch		
SECTION Chemistry Section		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The effects of <u>lymphocyte function</u> following brief exposure of <u>mouse spleens</u> to 2,3,7,8-tetrachlorodibenzodioxin (TCDD) in dimethylsulfoxide (DMSO) were studied. <u>DNA, RNA and protein synthesis</u> was severely <u>inhibited</u> at concentrations less than 10^{-7} mM TCDD per ml of DMSO when compared to DMSO treated controls. This concentration of TCDD accounted for ~0.14 ng TCDD uptake per spleen. The structurally related chemicals 3,4,3',4'-tetrachlorobiphenyl and NH ₂ -2,3,7-trichlorodibenzo-p-dioxin did not show any significant lymphocytic effects even at 2-fold higher concentrations. The ability of lymphocyte mitogens to bind to cell surface receptors was not effected by TCDD treatment. Studies with mitogens specific for lymphocyte subpopulations suggested that T-lymphocytes are slightly more susceptible to TCDD-induced immunosuppression than B-lymphocytes. Treatment with DMSO alone was slightly toxic to lymphoid cells as indicated by a 10-20% loss of cell viability within 4 hrs after DMSO exposure. While this made it somewhat difficult to determine TCDD-induced cytotoxicity, it appears that TCDD was slightly cytolytic to lymphocytes particularly 48 hrs after exposure.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Spleens from female B₆C₃F₁ mice were aseptically removed and submerged in various concentrations of ³TCDD, 3,4,3',4'-tetrachlorobiphenyl, or 2,3,7,8-TricDD for 10 sec and rinsed in RPMI 1640 medium. Controls were immersed in DMSO alone. DNA, RNA and protein synthesis was determined in vitro on cell cultures which were stimulated with either the T-cell mitogens, PHA or Con A or the B-cell mitogen LPS. Synthesis was determined by incorporation of either ³H-thymidine, ¹⁴C-leucine or ³H-uridine. TCDD uptake was determined on whole spleens with ¹⁴C-TCDD. Con A binding was determined by employing ³H-Con A. Viability was assessed by trypan blue exclusion.

MAJOR FINDINGS AND PROPOSED COURSE: These studies indicate that TCDD directly effects lymphocytic function. This could be related to the immunosuppression resulting from exposure to TCDD in laboratory animals.

The following studies are planned: (1) EM of treated spleens; (2) In vivo treatment of spleens with TCDD; (3) Effects of in vitro treatment with 2,3,7,8-tetrachlorodibenzofuran (TCDF); and (4) Localization of TCDD in the spleen by autoradiography with ³H-TCDD and/or fluorescence with fluorescein isothiocyanate (FITC)-conjugated antisera to TCDD.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Although the toxic effects of TCDD exposure in humans and animals has been extensively studied, little is known concerning the mechanisms of TCDD toxicity. Attempts to study the direct cytotoxic action of TCDD have been hampered due to the insolubility of TCDD in physiological buffers. This experimental approach allows one to study the direct effects, if any, of TCDD on cell function and possibly determine the primary action of TCDD on the cell.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30063-01 EBCB												
PERIOD COVERED October 1, 1977 to September 30, 1978														
TITLE OF PROJECT (80 characters or less) Molecular Orbital Calculations on Molecules of Environmental Interest														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT														
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">C. E. Parker</td> <td style="width: 35%;">Research Chemist</td> <td style="width: 15%;">EBCB NIEHS</td> </tr> <tr> <td>OTHER:</td> <td>J. R. Hass</td> <td>Senior Staff Fellow</td> <td>EBCB NIEHS</td> </tr> <tr> <td></td> <td>M. M. Bursey</td> <td>Visiting Scientist</td> <td>EBCB NIEHS</td> </tr> </table>			PI:	C. E. Parker	Research Chemist	EBCB NIEHS	OTHER:	J. R. Hass	Senior Staff Fellow	EBCB NIEHS		M. M. Bursey	Visiting Scientist	EBCB NIEHS
PI:	C. E. Parker	Research Chemist	EBCB NIEHS											
OTHER:	J. R. Hass	Senior Staff Fellow	EBCB NIEHS											
	M. M. Bursey	Visiting Scientist	EBCB NIEHS											
COOPERATING UNITS (if any) None														
LAB/BRANCH Environmental Biology and Chemistry Branch														
SECTION Chemistry Section														
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, NC 27709														
TOTAL MANYEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0.0												
CHECK APPROPRIATE BOX(ES)														
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER														
☐ (a1) MINORS ☐ (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords)														
<u>Molecular orbital calculations</u> have been done to determine <u>minimum energy configurations</u> of <u>neutral molecules</u> and <u>positive and negative ions</u> with respect to the mass spectral analysis of compounds of environmental interest.														

PROJECT DESCRIPTION

METHODS EMPLOYED: Programs were obtained from the Quantum Chemistry Program Exchange. These programs were modified as needed and run on the IBM 360/70 at the Triangle Universities Computation Center.

MAJOR FINDINGS AND PROPOSED COURSE: The INDO/CNDO/2 and MINDO/3 methods have been tried on the molecules of interest, after the programs were modified to handle the large number of atoms and the types of atoms needed for the study.

Preliminary results seemed to indicate that the INDO/CNDO/2 method may give invalid results due to the overestimate of non-bonding interactions. This project will be continued by investigating the use of MINDO/3 for these calculations.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Mass spectrometry is widely used for the identification and analysis of compounds of environmental interest. Mass spectrometry allows one to deduce structure through spectral interpretation, even where no reference compound is available. Correct interpretation of the data depends on the understanding of the gaseous ion chemistry involved. Molecular orbital theory allows the calculation of minimum energy configurations and charge distributions which are required for a complete understanding of fragmentation reaction pathways.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30064-01 EBCB						
PERIOD COVERED October 1, 1977 to September 30, 1978								
TITLE OF PROJECT (80 characters or less) Field Desorption Mass Spectrometry Studies								
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%;"> <tr> <td style="width: 33%;">PI: J. R. Hass</td> <td style="width: 33%;">Senior Staff Fellow</td> <td style="width: 33%;">EBCB NIEHS</td> </tr> <tr> <td>OTHER: M. M. Bursey</td> <td>Visiting Scientist</td> <td>EBCB NIEHS</td> </tr> </table>			PI: J. R. Hass	Senior Staff Fellow	EBCB NIEHS	OTHER: M. M. Bursey	Visiting Scientist	EBCB NIEHS
PI: J. R. Hass	Senior Staff Fellow	EBCB NIEHS						
OTHER: M. M. Bursey	Visiting Scientist	EBCB NIEHS						
COOPERATING UNITS (if any) R. Shapiro, Dept. of Chemistry, New York University								
LAB/BRANCH Environmental Biology and Chemistry Branch SECTION Chemistry Section INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709								
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.3	OTHER: 0.2						
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS								
SUMMARY OF WORK (200 words or less - underline key words) <u>Adducts of phosphodiesterases and polynucleotides with N-acetoxy-2-acetamido-fluorene were prepared for analysis by field desorption mass spectrometry. Similar adducts of 9-bromomethylbenz(a)anthracene and benz(a)pyrene epoxydihydrodiol will be examined as well.</u>								

PROJECT DESCRIPTION

METHODS EMPLOYED: Field desorption mass spectrometry, high performance liquid chromatography.

MAJOR FINDINGS AND METHODS EMPLOYED: The major adduct products from reaction of N-acetoxy-2-acetamidofluorene (NA-AAF) with phosphodinucleotides and small polynucleotides in citrate buffer were separated and isolated. As soon as the field desorption capability of the ZAB-2F mass spectrometer is routinely operational, these adducts will be used as models to find appropriate conditions for obtaining the field desorption mass spectra of small polynucleotide-NA-AAF adducts. Four adducts of 9-bromomethylbenz(a)anthracene with nucleosides have been obtained from Professor Robert Shapiro (New York University) and phosphodinucleotide adducts of benzo(a)pyrene epoxydiol will be prepared following successful analysis of the NA-AAF adducts.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Finding mass spectrometric methods for the analysis of small polynucleotide-carcinogen adducts will permit a rapid method for identification of these compounds, permitting studies of the effects of neighboring groups upon the reactivity of the site attacked. Such studies should illuminate the micro-environments important in environmental chemical carcinogenesis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30065-01 EBCB																
PERIOD COVERED October 1, 1977 to September 30, 1978																		
TITLE OF PROJECT (80 characters or less) Studies of Mass Spectral Reactions in Field-Free Regions																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">J. R. Hass</td> <td style="width: 35%;">Senior Staff Fellow</td> <td style="width: 15%;">EBCB NIEHS</td> </tr> <tr> <td>OTHER:</td> <td>M. M. Bursey</td> <td>Visiting Scientist</td> <td>EBCB NIEHS</td> </tr> <tr> <td></td> <td>L. A. Levy</td> <td>Research Chemist</td> <td>EBCB NIEHS</td> </tr> <tr> <td></td> <td>P. W. Albro</td> <td>Research Chemist</td> <td>EBCB NIEHS</td> </tr> </table>			PI:	J. R. Hass	Senior Staff Fellow	EBCB NIEHS	OTHER:	M. M. Bursey	Visiting Scientist	EBCB NIEHS		L. A. Levy	Research Chemist	EBCB NIEHS		P. W. Albro	Research Chemist	EBCB NIEHS
PI:	J. R. Hass	Senior Staff Fellow	EBCB NIEHS															
OTHER:	M. M. Bursey	Visiting Scientist	EBCB NIEHS															
	L. A. Levy	Research Chemist	EBCB NIEHS															
	P. W. Albro	Research Chemist	EBCB NIEHS															
COOPERATING UNITS (if any) None																		
LAB/BRANCH Environmental Biology and Chemistry Branch																		
SECTION Chemistry Section																		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																		
TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.4	OTHER: 0.2																
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																		
SUMMARY OF WORK (200 words or less - underline keywords) <u>Unimolecular mass-analyzed ion kinetic energy spectra</u> of model polychlorinated biphenyls were analyzed with respect to the type of <u>fragmentation</u> which controls the <u>kinetic energy release</u> in the major decompositions of the molecular ion, giving new correlations of structure with spectral fragmentation behavior. <u>Collision-induced decompositions of M⁺ ions</u> produced in chemical ionization of <u>aliphatic esters</u> and small C_nH_{2n+1}O⁺ ions (from collision-induced charge reversal of alkoxide ions) were studied as models for the amount of analytically useful information available by induction of collisions in the mass spectrometer. Collision-induced dissociations lead to additional analytical information from the mass spectra of <u>Di-2-ethylhexylphthalate metabolites</u>.																		

PROJECT DESCRIPTION

METHODS EMPLOYED: High resolution mass spectrometry with metastable scanning.

MAJOR FINDINGS AND PROPOSED COURSE: Pure metastable mass spectra were obtained for a number of simple polychlorinated biphenyls. Analysis of the ion kinetic energy spectra of the major peaks in their mass spectra allowed the classification of the kinetic energy releases into categories characteristic of the substitution pattern. Further, it was possible to establish orders of precedence in PCBs with complex substitution patterns so that the relative influence of these types of substitution on the ion kinetic energy spectra was determined. Loss of chlorine from PCB ions was studied in some cases until no more chlorine remained in the ion, and the structure of the remaining hydrocarbon ion was then compared with classical models.

This method of spectroscopy is so new that there is no library of data available for comparison of spectra; it is important to collect such spectra since it has been proposed that ion kinetic energy spectra of ions will compliment gas chromatography/mass spectrometry, at least to some extent. To this end we established fragmentation patterns for the analysis of ester ions produced in chemical ionization conditions and examined the behavior of organic anions undergoing charge reversal in the mass spectrometer on collision with small gas molecules. Alkoxide ions have been examined and found to give spectra easily correlated with structure.

The methane positive ion chemical ionization mass spectrum of each metabolite of di-2-ethylhexylphthalate contains an ion which corresponds to the partially oxidized side-chain. By tuning this ion on the collision cell of our reversed-sector mass spectrometer and scanning the electric sector to obtain the energy spectrum of the fragment ions, data were obtained which correlated very well with compound structure in most cases.

This project will be continued by completing the above experiments. In addition, these techniques will be extended to other environmental chemicals.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Mass spectrometry (in combination with gas chromatography) is the most sensitive and specific analytical method presently available for dealing with complex samples of environmental/biological origins. The results of this project will increase our understanding of the fundamentals of the technique as well as provide more specific information for structured analysis.

PUBLICATIONS

Hass, J.R., Bursey, M.M., and Levy, L.A.: Influence of symmetrically placed substituents on kinetic energy release during loss of chlorine from chlorinated biphenyls. Org. Mass Spectrom. (in press)

Hass, J.R., Bursey, M.M., Levy, L.A., and Harvan, D.J.: Kinetic energy release during loss of chlorine from polychlorinated biphenyls. Competition between reactions characteristic of different substituent sites. Org. Mass Spectrom. (in press)

Bursey, M.M., Harvan, D.J., and Hass, J.R.: $C_{12}H_8^+$ structure and formation studied by mass-analyzed ion kinetic energy spectrometry. Org. Mass Spectrom. (in press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30066-01 EBCB						
PERIOD COVERED October 1, 1977 to September 30, 1978								
TITLE OF PROJECT (80 characters or less) Development of Synthetic Methods for Polyhalogenated Aromatics								
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%;"> <tr> <td style="width: 33%;">PI: J.D. McKinney</td> <td style="width: 33%;">Supervisory Research Chemist</td> <td style="width: 33%;">EBCB NIEHS</td> </tr> <tr> <td>OTHER: A. Norstrom</td> <td>Guest Worker</td> <td>EBCB NIEHS</td> </tr> </table>			PI: J.D. McKinney	Supervisory Research Chemist	EBCB NIEHS	OTHER: A. Norstrom	Guest Worker	EBCB NIEHS
PI: J.D. McKinney	Supervisory Research Chemist	EBCB NIEHS						
OTHER: A. Norstrom	Guest Worker	EBCB NIEHS						
COOPERATING UNITS (if any) None								
LAB/BRANCH Environmental Biology and Chemistry Branch								
SECTION Chemistry Section								
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709								
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0.0						
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS								
SUMMARY OF WORK (200 words or less - underline keywords) The Pd(II)-acetate catalyzed ring closure of <u>halogenated diphenyl</u> <u>ethers and benzophenones</u> is being utilized to synthesize <u>halogenated</u> <u>dibenzofurans and fluorenones</u> . A polybrominating agent, <u>dibromoisocyanuric</u> <u>acid</u> , is used to obtain certain polybrominated naphthalenes and biphenylenes. Such polyhalogenated aromatic compounds are of current toxicological interest as potential persistent environmental agents.								

PROJECT DESCRIPTION

METHODS EMPLOYED: Synthetic techniques, organometallic reagents, functional group transformations. Mass spectroscopy, other spectroscopic methods (IR, NMR), chromatography (column, glc, liquid).

MAJOR FINDINGS AND PROPOSED COURSE: A number of chlorinated diphenyl ethers have been synthesized. These together with some chlorinated dibenzophenones will be ring closed to the corresponding dibenzofurans and fluorenones when the high-hazard laboratory is available. The polybrominating agent, dibromoisocyanuric acid, is being tested for the synthesis of highly brominated naphthalenes and biphenylenes.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: There is still a great need for simple synthetic routes to many polyhalogenated aromatics. This project will, hopefully, give simple synthetic routes to many toxicologically interesting polyhalogenated aromatics which are known to persist in the environment.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30067-01 EBCB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Characterization of the Binding of Heavy Metals in Biological Systems		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: R.H. Cox Research Chemist EBCB NIEHS OTHER: None		
COOPERATING UNITS (if any) None		
LAB/BRANCH Environmental Biology and Chemistry Branch		
SECTION Chemistry Section		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) ^1H, ^{13}C and metal nuclear magnetic resonance (NMR) spectroscopy is being used to investigate the <u>specific binding</u> character of certain heavy metals using purified biopolymers, their monomer and other isolated and definable biological systems as models. An attempt is being made to correlate binding propensity with toxic effects of the heavy metals. Early work indicates that ^{209}Pb NMR offers a useful probe into the binding of lead.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Multi nuclei nuclear magnetic resonance (NMR) spectroscopy using biological models for studying the interactions between heavy metals. Isolation and characterization of specific binding site(s) in body tissue and fluid.

MAJOR FINDINGS AND PROPOSED COURSE: There is an increasing body of evidence that the transport and accumulation of heavy metals in body tissue and fluids is related to the binding of the metals in biological systems. Studies to date have shown that ²⁰⁷Pb NMR offers a very sensitive probe into the binding of lead. Future studies should include binding of lead to various biological systems with an attempt to correlate binding propensity with toxicity. In addition, NMR methods should be developed for investigating the binding of other heavy metals.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

There is increasing evidence that certain heavy metals produced as energy by-products may accumulate in body tissue and fluid and present serious health effects. Binding propensity of the heavy metals and toxicity and accumulation may be correlatable. An understanding of the specific molecular level interactions involved in binding may permit one to predict, prevent, or reverse them.

PUBLICATIONS

Cox, R.H.: Carbon-13 and lead-207 NMR studies of organolead compounds. J. Magnetic Res. (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30068-01 EBCB												
PERIOD COVERED October 1, 1977 to September 30, 1978														
TITLE OF PROJECT (80 characters or less) Effects of Chronic Low Level Lead Exposure in Rats. Immune Alterations.														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT														
<table border="0"> <tr> <td>PI:</td> <td>M. I. Luster</td> <td>Staff Fellow</td> <td>EBCB NIEHS</td> </tr> <tr> <td>OTHER:</td> <td>C. A. Kimmel</td> <td>Senior Staff Fellow</td> <td>LET NIEHS</td> </tr> <tr> <td></td> <td>R. Faith</td> <td>Staff Fellow</td> <td>EBCB NIEHS</td> </tr> </table>			PI:	M. I. Luster	Staff Fellow	EBCB NIEHS	OTHER:	C. A. Kimmel	Senior Staff Fellow	LET NIEHS		R. Faith	Staff Fellow	EBCB NIEHS
PI:	M. I. Luster	Staff Fellow	EBCB NIEHS											
OTHER:	C. A. Kimmel	Senior Staff Fellow	LET NIEHS											
	R. Faith	Staff Fellow	EBCB NIEHS											
COOPERATING UNITS (if any) None														
LAB/BRANCH Environmental Biology and Chemistry Branch														
SECTION Chemistry Section														
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709														
TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.1	OTHER: 0.1												
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords) Studies were performed to investigate the effects of <u>chronic, low level</u> pre- and postnatal lead exposure on immune functions in rats. Weanling female rats were exposed to lead (as lead acetate) in their drinking water at 0, 25, and 50 ppm for 7 weeks. At the end of 7 weeks they were mated with untreated males and continued on the same dosage throughout gestation and lactation. The offspring of these females were weaned at 21 days of age and continued on the same lead exposure regimen as their mothers. These offspring were used in immune surveillance procedures between 35 and 45 days of age. Lead exposure at the levels employed had no statistically significant effect on growth and did not result in overt signs of toxicity. <u>Thymic weights</u> were significantly decreased in both males and females of the two lead dosage groups. Furthermore, lead exposure resulted in suppression of responsiveness of <u>lymphocytes</u> to mitogen stimulation and in reduced delayed hypersensitivity responsiveness. A marked depression in the antibody response to sheep red blood cells (SRBC) as well as decreased serum IgG levels were observed. Serum IgM and IgA levels were normal.														

PROJECT DESCRIPTION

METHODS EMPLOYED: Cell mediated functions were assessed in vitro by the response of splenic cell cultures to the mitogens Con A and PHA and in vivo by the delayed hypersensitivity reaction to purified protein derivative. Humoral immunity was determined by the antibody plaque assay and serum IgG levels.

MAJOR FINDINGS AND PROPOSED COURSE: Results from these studies confirm earlier studies that lead suppresses antibody responses to T-dependent antigens. In addition, we found that lead also induces a profound cell mediated immune suppression as indicated by depressed responses to T-cell mitogen stimulation and decreased DHRs. The fact that the antibody response to LPS, a T-independent antigen, was not altered suggested that the T-lymphocyte and not the B-lymphocyte is affected by lead exposure. No future studies have been planned.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Additional significance is lent to these results when viewed in light of the blood lead levels obtained with the lead exposure regimens employed in this and other studies. Blood lead determinations indicated that the levels seen with the 25 and 50 ppm doses employed here were comparable to blood lead levels found in many children in urban areas. In light of these facts, low-level lead exposure may be more undesirable than previously thought, since it is conceivable that it leads to immunosuppression in humans as well as in rats as demonstrated here.

PUBLICATIONS

Luster, M.I., Faith, R.E., and Kimmel, C.A.: Depression of humoral immunity in rats following chronic developmental lead exposure. J. Environ. Path. Toxicol. (in press).

Faith, R.E., Luster, M.I., and Kimmel, C.A.: Cell mediated immune suppression in rats following chronic developmental lead exposure. Clin. Exptl. Immunol. (in press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30069-01 EBCB						
PERIOD COVERED October 1, 1977 to September 30, 1978								
TITLE OF PROJECT (80 characters or less) The Effects of Adding Pentachlorophenol (Penta) to the Diet of Heifers. Immune Function Studies.								
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%;"> <tr> <td style="width: 33%;">PI: M. I. Luster</td> <td style="width: 33%;">Staff Fellow</td> <td style="width: 33%;">EBCB NIEHS</td> </tr> <tr> <td>OTHER: E. E. McConnell</td> <td>Veterinary Pathologist</td> <td>EBCB NIEHS</td> </tr> </table>			PI: M. I. Luster	Staff Fellow	EBCB NIEHS	OTHER: E. E. McConnell	Veterinary Pathologist	EBCB NIEHS
PI: M. I. Luster	Staff Fellow	EBCB NIEHS						
OTHER: E. E. McConnell	Veterinary Pathologist	EBCB NIEHS						
COOPERATING UNITS (if any) None								
LAB/BRANCH Environmental Biology and Chemistry Branch								
SECTION Chemistry Section								
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709								
TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.1	OTHER: 0.1						
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS								
SUMMARY OF WORK (200 words or less - underline keywords) Pentachlorophenol (PCP), a widely used wood preservative, may contain unwanted toxic contaminants such as chlorinated dibenzo-p-dioxin and dibenzofurans. The results of a recent investigation into the cause of a toxic syndrome in dairy animals suggests that dioxins and dibenzofurans (and in turn, PCP) may have played a role. The immunosuppression induced by 2,3,7,8-TCDD and 2,3,7,8-TCDF in laboratory animals suggested that immune alterations may be induced in these cattle. The cattle in this study consisted of 15 (12-15 months old) female Holstein cattle. The compound was administered in the feed to achieve the desired dose. The cattle were randomly subdivided into 5 groups of 3 each and given the following doses for 120 days - GROUP A: Maximum tolerated dose of pure PCP; GROUP B: Same dose of commercial PCP; GROUP C: 1/3 dose B and 2/3 of pure PCP; GROUP D: 1/3 dose C and 9 times equal amount of pure PCP; GROUP E: Nontreated controls.								

PROJECT DESCRIPTION

METHODS EMPLOYED: Immune surveillance studies were performed on days 0, 45, 90 and 120 of dosing. Cell mediated immunity was assessed in vitro by the response of isolated peripheral blood lymphocytes to the mitogens Con A and PHA and to response of heterologous lymphocytes in mixed lymphocyte culture reactions. Alterations in humoral immunity was assessed by antibody responses to SRBC, quantitation of IgG, IgM, IgA and C3 serum levels, and production of anti-nuclear antibody by immunofluorescence. Corticosteroid levels will be determined by radioimmunoassay.

MAJOR FINDINGS AND PROPOSED COURSE: The data has not been statistically analyzed so results can not be evaluated as yet. The proposed course is unknown at the present.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Environmental contaminants may exert subtle effects on man and animals. Among these effects may be a modulation of immune response. It is important to establish which environmental chemicals may induce modulation in order to evaluate the hazards of immune alterations in man and animals.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30070-01 EBCB									
PERIOD COVERED October 1, 1977 to September 30, 1978											
TITLE OF PROJECT (80 characters or less) Effect of <u>In Utero</u> Exposure to Diethylstilbestrol. Immune Response Modulation in Mice.											
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: M. I. Luster</td> <td style="width: 33%;">Staff Fellow</td> <td style="width: 33%;">EBCB NIEHS</td> </tr> <tr> <td>OTHER: J. A. McLachlan</td> <td>Developmental Toxicologist</td> <td>LET NIEHS</td> </tr> <tr> <td>R. Faith</td> <td>Staff Fellow</td> <td>EBCB NIEHS</td> </tr> </table>			PI: M. I. Luster	Staff Fellow	EBCB NIEHS	OTHER: J. A. McLachlan	Developmental Toxicologist	LET NIEHS	R. Faith	Staff Fellow	EBCB NIEHS
PI: M. I. Luster	Staff Fellow	EBCB NIEHS									
OTHER: J. A. McLachlan	Developmental Toxicologist	LET NIEHS									
R. Faith	Staff Fellow	EBCB NIEHS									
COOPERATING UNITS (if any) None											
LAB/BRANCH Environmental Biology and Chemistry Branch											
SECTION Chemistry Section											
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709											
TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.1	OTHER: 0.1									
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS											
SUMMARY OF WORK (200 words or less - underline keywords) The effect of <u>in utero exposure to diethylstilbestrol (DES)</u> on the <u>immune system</u> was investigated. Mice were exposed to DES through maternal dosing (0.1 mg/animal) on day 16 of gestation. Parameters of cell-mediated and humoral immune function were evaluated between 7 and 9 weeks of age. DES suppressed cell-mediated immune function in both males and particularly females. In contrast a <u>sex interaction</u> was noted in humoral immune function with enhanced response in males and either no effect or slight suppression in females.											

PROJECT DESCRIPTION

METHODS EMPLOYED: Cell mediated functions were assessed in vitro by the response of splenic cell cultures to the mitogen Con A and in vivo by the delayed hypersensitivity reaction to purified protein derivative (PPD). Humoral immune functions were assayed by the antibody plaque assay to both a T-dependent and T-independent antigen. Serum IgG1, IgG2, IgM and IgA levels were determined by radial immunodiffusion analysis.

MAJOR FINDINGS AND PROPOSED COURSE: These studies indicated that in utero exposure to DES can alter immunocompetence in mice. DES suppressed cell-mediated immune functions both in males and particularly females as evidenced by decreased responses to Con A stimulation and suppressed DHR's. In contrast a sex interaction was noted in humoral immune function with enhanced antibody response to LPS (T-independent) in males and decreased antibody responses in females. No further experiments are planned at this time.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: For many agents the prenatal organism is more sensitive to chemical injury than the corresponding adult. In this respect, it was reported that among young females with clear-cell adenocarcinoma of the vagina there was a significantly increased use of maternal DES during pregnancy compared with mothers of control subjects. It should be noted that the immune surveillance theory against tumor development is gaining wide support.

PUBLICATIONS

Luster, M.I., Faith, R.E., and McLachlan, J. A. Modulation of the antibody response following in utero exposure to diethylstilbestrol. Bull. Environ. Contam. Toxicol. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30071-01 EBCB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Effects of 2,3,7,8-Tetrachlorodibenzofuran (TCDF) on the Immunocompetence in Guinea Pigs and Immune Modulation by Other Environmental Chemicals		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: M. I. Luster OTHER: None Staff Fellow EBCB NIEHS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Environmental Biology and Chemistry Branch		
SECTION Chemistry Section		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.1	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) These studies were undertaken to investigate the effects of exposure to <u>2,3,7,8-Tetrachlorodibenzofuran (TCDF)</u> on <u>immunocompetence in guinea pigs</u>. Guinea pigs were orally given 6 doses of either 1.0, 0.5, 0.17 or 0.05 μg of TCDF/Kg body weight in corn oil weekly over a 6 week period. Controls received corn oil alone. These studies indicated that <u>cell mediated immune functions</u> was <u>suppressed</u> at the higher dose levels, particularly at 1.0 μg/Kg dose.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Cell mediated immunity (T-cell) was assessed in vitro by the response of splenic lymphocytes to the mitogens Con A and PHA and the production of macrophage inhibition factor. Cell mediated immunity was assessed in vivo by delayed hypersensitivity reactions. Humoral immunity (B-cell) was assessed in vivo by the antibody response to bovine gamma globulin and quantitation of serum IgG levels by radial immunodiffusion and in vitro by the mitogenic response of lymphocytes to LPS.

MAJOR FINDINGS AND PROPOSED COURSE: Results from these studies suggested that TCDF induces immune suppression as indicated by suppressed Con A, PHA and LPS lymphocyte responses in the 1.0 and 0.5 $\mu\text{g/Kg}$ dosage groups. DHR's and production of macrophage inhibition factor were also decreased in these groups. Serum IgG levels and antibody responses appeared normal. Thymus/body weight ratios were decreased in the 1.0 $\mu\text{g/Kg}$ group.

Future planned studies with TCDF will involve effects of in vitro exposure on lymphocytic function.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Environmental contaminants may exert subtle effects on man and animals. Among these effects may be a modulation of immune response. It is important to establish which environmental chemicals may induce modulation of immune responses and their mode of action, in order to evaluate the hazards of immune modulation in man and animals.

PUBLICATIONS

Faith, R.E., Luster, M.I., and Moore, J.A.: Chemical separation of helper cell function. Cell. Immunol. (in press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30072-01 EBCB									
PERIOD COVERED October 1, 1977 to September 30, 1978											
TITLE OF PROJECT (80 characters or less) Effects of Polybrominated Biphenyls. Immune Response in Rodents.											
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: M. I. Luster</td> <td style="width: 33%;">Staff Fellow</td> <td style="width: 33%;">EBCB NIEHS</td> </tr> <tr> <td>OTHER: J. A. Moore</td> <td>Supervisory Veterinary Medical Officer</td> <td>EBCB NIEHS</td> </tr> <tr> <td>R. E. Faith</td> <td>Staff Fellow</td> <td>EBCB NIEHS</td> </tr> </table>			PI: M. I. Luster	Staff Fellow	EBCB NIEHS	OTHER: J. A. Moore	Supervisory Veterinary Medical Officer	EBCB NIEHS	R. E. Faith	Staff Fellow	EBCB NIEHS
PI: M. I. Luster	Staff Fellow	EBCB NIEHS									
OTHER: J. A. Moore	Supervisory Veterinary Medical Officer	EBCB NIEHS									
R. E. Faith	Staff Fellow	EBCB NIEHS									
COOPERATING UNITS (if any) None											
LAB/BRANCH Environmental Biology and Chemistry Branch											
SECTION Chemistry Section											
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709											
TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.1	OTHER: 0.1									
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS											
SUMMARY OF WORK (200 words or less - underline keywords) Studies were performed to investigate the effects of <u>Firemaster FF-1</u>, a chemical fire retardant consisting of a mixture of <u>polybrominated biphenyls (PBB)</u>, on <u>immune functions</u> in mice and rats. Animals received 22 daily treatments of either 0.03, 0.3, 3.0 or 30 mg PBB/kg body weight in a period covering 30 days. PBB exposure severely depressed <u>cell mediated immunity</u> in both mice and rats at the higher dosage levels as indicated by depressed responsiveness of splenic lymphocytes to mitogenic stimulation by polyclonal T-cell activators. Additionally, <u>humoral immunity</u> was depressed in mice at the 30.0 ppm dosage level. Assays for humoral immune functions included antibody production, serum immunoglobulin levels, and mitogenic stimulation of splenic lymphocytes to a polyclonal B-cell activator. These studies indicate that PBB exposure can lead to suppression of both humoral and particularly cell mediated immune responses.											

PROJECT DESCRIPTION

METHODS EMPLOYED: Cell mediated (T-cell) immunity was assessed in vitro by the response of splenic cell cultures to the mitogens Con A and PHA. Humoral immunity was assessed by the response of splenic cell cultures to the mitogens E. coli 055 B5 LPS and PWM and by plaque forming cells to T-dependent antigens. Serum immunoglobulin IgG, IgM and IgA levels were quantitated by radial immunodiffusion in mice.

MAJOR FINDINGS AND PROPOSED COURSE: Both mice and rats exposed to the highest dose level of Firemaster revealed an absolute and relative decrease in spleen and thymus weights. Splenic responses to the T-cell mitogens were decreased in both mice and rats at the 30.0 and 3.0 mg/kg dosage groups. Response to the B-cell mitogens were less effected as were antibody responses. Serum IgG1 and IgM levels were decreased in mice exposed to the 30 mg/kg dosage level only.

A 6 month chronic feeding study is underway. In addition to repeating earlier studies, we will expand our studies to include delayed hypersensitivity reactions, mixed lymphocyte reaction and in mice only quantitation of B- and T-cells. Serum cortisone levels will also be determined.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It has been reported that among the toxicological effect from individuals exposed to PBB in the state of Michigan was a suppression of immunocompetence. It is important to confirm these studies under controlled conditions in a laboratory environment.

PUBLICATIONS

Luster, M.I., Faith, R.E., and Moore, J.A. Effects of polybrominated biphenyls on immune response in rodents. Environ. Health Perspect. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30073-01 EBCB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (60 characters or less) Efforts to Enhance the Elimination of 2,3,7,8-Tetrachloro-dibenzo-p-dioxin from the Body of Rats		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: OTHER:	E.E. McConnell H.B. Matthews J.A. Moore M.W. Harris	Veterinary Pathologist Research Chemist Supervisory Veterinary Medical Officer Biological Laboratory Technician EBCB NIEHS LPK NIEHS EBCB NIEHS EBCB NIEHS
COOPERATING UNITS (if any) None		
LAB/BRANCH Environmental Biology and Chemistry Branch		
SECTION Comparative Pathology Section		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.3	PROFESSIONAL: 0.2	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The object of this study was to determine if <u>cholestyrimine, mineral oil</u> or charcoal had any effect on enhancing the <u>rate of elimination</u> of <u>2,3,7,8-Tetrochlorodibenzo-p-dioxin (TCDD)</u> from the <u>body of rats</u> .		

PROJECT DESCRIPTION

METHODS EMPLOYED: Fischer strain female rats were given diets containing either cholestyrimine, mineral oil or charcoal. (^{14}C) TCDD was injected intraperitoneally either 7 days before or 7 days after the rats were placed on the diets. Radioactivity was monitored in the feces and urine for 14 days after TCDD exposure. Various tissues obtained at necropsy were also evaluated for radioactivity.

MAJOR FINDINGS: Cholestyrimine and mineral oil did not enhance elimination of TCDD from the body of rats. However, charcoal dramatically enhanced elimination and reduced body burden if used prior to TCDD exposure but had little effect if given 7 days post exposure. This project is to be continued.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Chlorinated dibenzodioxins are extremely toxic compounds which may find their way into the environment via contamination of several useful chemicals. Significant human exposures have occurred in many areas of the world. Since these exposures are usually of a chronic (prolonged) nature it would be extremely useful to find a therapeutic compound which would both reduce absorption and enhance elimination of these chemicals from the body.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30074-01 EBCB																
PERIOD COVERED October 1, 1977 to September 30, 1978																		
TITLE OF PROJECT (80 characters or less) A Study of Japanese Rice (Yusho) Oil in Guinea Pigs																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																		
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 45%;">E.E. McConnell</td> <td style="width: 30%;">Veterinary Pathologist</td> <td style="width: 10%;">EBCB NIEHS</td> </tr> <tr> <td>OTHER:</td> <td>M. Morita</td> <td>Visiting Chemist</td> <td>EBCB NIEHS</td> </tr> <tr> <td></td> <td>J.D. McKinney</td> <td>Supervisory Research Chemist</td> <td>EBCB NIEHS</td> </tr> <tr> <td></td> <td>M.W. Harris</td> <td>Biological Laboratory Tech.</td> <td>EBCB NIEHS</td> </tr> </table>			PI:	E.E. McConnell	Veterinary Pathologist	EBCB NIEHS	OTHER:	M. Morita	Visiting Chemist	EBCB NIEHS		J.D. McKinney	Supervisory Research Chemist	EBCB NIEHS		M.W. Harris	Biological Laboratory Tech.	EBCB NIEHS
PI:	E.E. McConnell	Veterinary Pathologist	EBCB NIEHS															
OTHER:	M. Morita	Visiting Chemist	EBCB NIEHS															
	J.D. McKinney	Supervisory Research Chemist	EBCB NIEHS															
	M.W. Harris	Biological Laboratory Tech.	EBCB NIEHS															
COOPERATING UNITS (if any) None																		
LAB/BRANCH Environmental Biology and Chemistry Branch																		
SECTION Comparative Pathology Section																		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																		
TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.3	OTHER: 0.1																
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																		
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of the study is to determine the relative degree of toxicity (oral LD ₅₀) in the guinea pig and define the target organ/lesions produced by Yusho oil (rice oil contaminated with PCB's, furans, etc). Subsequently, to determine the toxicity of the contaminants (by classes) to determine which one(s) are responsible for the toxic properties of the Yusho oil.																		

PROJECT DESCRIPTION

METHODS EMPLOYED: Hartley strain guinea pigs were given single oral (via gavage) doses of Yusho oil (actual oil from "Yusho" disaster) in order to study the toxicologic, pathologic and clinical pathologic effects of the intoxication.

MAJOR FINDINGS: The acute LD₅₀ in the guinea pig is about 1.6 ml of contaminated rice (Yusho) oil/kg. The toxicopathology is essentially the same as that observed with PCB's, furans, dioxins and naphthalenes, i.e., thymic atrophy, increased liver weight, and hemorrhage in the adrenal medulla. The fractions of the rice oil have not been studied as of 1 April 1978. The project is to be continued.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The disaster in Japan caused by contamination of rice oil used in cooking is well known and described. PCB's were originally thought to be the sole contaminant. Many of the people showing clinical signs of "Yusho" disease are not improving although PCB levels in their tissues are decreasing. It is important to determine if other toxic factors are present in the rice oil at levels high enough to explain the continuing problem.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30075-01 EBCB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) The Comparative Toxicity of Technical vs Pure Pentachlorophenol in Dairy Cattle		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: E.E. McConnell OTHER: J.A. Moore B.N. Gupta C. Parker M. Luster J. Goldstein A. Rakes R.E. Wilson F.A. Talley	Veterinary Pathologist Senior Veterinary Medical Officer Veterinary Pathologist Research Chemist Research Chemist Research Pharmacologist Animal Scientist Biological Laboratory Technician Histology Technician	EBCB NIEHS EBCB NIEHS EBCB NIEHS EBCB NIEHS EBCB NIEHS EBCB NIEHS NCSU EBCB NIEHS EBCB NIEHS
COOPERATING UNITS (if any) Animal Science Department, North Carolina State University, Raleigh, North Carolina		
LAB/BRANCH Environmental Biology and Chemistry Branch		
SECTION Comparative Pathology Section		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 1.7	PROFESSIONAL: 1.3	OTHER: 0.4
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this 150 day feeding study was to determine the effects of unwanted contaminants in <u>technical pentachlorophenol (PCP)</u> in dairy cattle by comparing them to pure PCP and non-exposed animals. Parameters studied included <u>clinical examination</u>, <u>weight gain</u>, <u>hoof growth</u>, <u>hematology</u> and <u>clinical chemistry</u>, <u>gross and histopathology</u>, <u>electron microscopy</u>, <u>immunology</u>, <u>hepatic enzyme induction</u>, and <u>chemical analysis</u> of tissues. Preliminary data indicate that the technical compound is significantly more toxic than pure PCP.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Purebred yearling female Holstein cattle were exposed to either pure or technical pentachlorophenol and combinations thereof in the feed for 150 days. Parameters studied during this period included clinical signs, feed consumption, weight gain, hoof growth, hematology and clinical chemistry, urinalysis and immunology. At the termination of the experiment the animals were necropsied and representative tissues were collected for histopathology, electron microscopy, hepatic enzyme induction evaluation, immunology, and chemical analysis.

MAJOR FINDINGS: Preliminary findings indicate that technical PCP is significantly more toxic than pure PCP, based on differences in weight gain, hematologic differences and clinical syndrome. Most of the other parameters have not been completed as of 1 April 1978. Project should be completed this FY.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM AT THE INSTITUTE
Pentachlorophenol is widely used as a wood preservative and fungistatic agent. In some cases it is contaminated with unwanted toxic chemicals such as dibenzo-p-dioxins. There have been clinical situations in which PCP treated wood has been incriminated as a etiologic factor in diseased dairy cattle in the north central United States. Because of this farm problem and the potential of contaminated food (beef) reaching humans, it is important to assess the toxicity of PCP in dairy cattle and to determine the importance of contaminants therein.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30076-01 EBCB												
PERIOD COVERED October 1, 1977 to September 30, 1978														
TITLE OF PROJECT (80 characters or less) The Comparative Toxicity in Guinea Pigs of Related Halogenated Polycyclic Hydrocarbons														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 30%;">E.E. McConnell</td> <td style="width: 30%;">Veterinary Pathologist</td> <td style="width: 30%;">EBCB NIEHS</td> </tr> <tr> <td></td> <td>J.A. Moore</td> <td>Senior Veterinary Medical Officer</td> <td>EBCB NIEHS</td> </tr> <tr> <td></td> <td>M.W. Harris</td> <td>Biological Laboratory Technician</td> <td>EBCB NIEHS</td> </tr> </table>			PI:	E.E. McConnell	Veterinary Pathologist	EBCB NIEHS		J.A. Moore	Senior Veterinary Medical Officer	EBCB NIEHS		M.W. Harris	Biological Laboratory Technician	EBCB NIEHS
PI:	E.E. McConnell	Veterinary Pathologist	EBCB NIEHS											
	J.A. Moore	Senior Veterinary Medical Officer	EBCB NIEHS											
	M.W. Harris	Biological Laboratory Technician	EBCB NIEHS											
COOPERATING UNITS (if any) None														
LAB/BRANCH Environmental Biology and Chemistry Branch														
SECTION Comparative Pathology Section														
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709														
TOTAL MANYEARS: 0.3	PROFESSIONAL: 0.1	OTHER: 0.2												
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this study was to compare the relative toxicity of <u>dibenzo-p-dioxin</u>, <u>dibenzofuran</u>, <u>polychlorinated biphenyl</u> and <u>chlorinated naphthalene</u> using the most toxic isomer of each in male guinea pigs. Toxicopathologic parameters included <u>clinical signs</u>, <u>weight gain</u>, <u>organ weight</u>, <u>necropsy examination</u> and <u>histopathology</u>.														

PROJECT DESCRIPTION

METHODS EMPLOYED: Male Hartley strain guinea pigs were given a single dose via gavage of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin, 2,3,7,8-Tetrachlorodibenzofuran, 3,3',4,4',5,5'-Hexachlorinated biphenyl and 2,3,6,7-Tetrachloronapthalene in order to determine their relative toxicity.

MAJOR FINDINGS: Based on lethality the relative order of toxicity is TCDD (most toxic) > TCDF >> HCB >> TCN. At the lethal dose the clinical signs, weight gain, organ weight effect and lesions are identical. Project is completed.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

The above chemicals are of major importance to environmental toxicology due to their widespread occurrence. There has been a great deal of confusion regarding their relative toxicity and comparative pathology which at least in the guinea pig is not well defined.

PUBLICATIONS

McConnell, E.E. and McKinney, J.D.: Exquisite Toxicity in the Guinea Pig to Structurally Similiar Halogenated Dioxins, Furans, Biphenyls and Napthalenes. Toxicol. Appl. Pharmacol. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30077-01 EBCB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Dose Response to the Carcinogens, 1,2-Dimethylhydrazine (DMH) and Methyl- azoxymethanol (MAM) in Rats		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: E. E. McConnell OTHER: J. A. Moore R. E. Wilson	Veterinary Pathologist Supervisory Veterinary Medical Officer Biological Laboratory Technician	EBCB NIEHS EBCB NIEHS EBCB NIEHS
COOPERATING UNITS (if any) J. Fawkes, Chemistry Section, EBCB		
LAB/BRANCH Environmental Biology and Chemistry Branch		
SECTION Comparative Biology Section		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 0.3	PROFESSIONAL: 0.2	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The "HEW Committee to Coordinate Toxicology and Other Related Programs" has designed a study to establish the biologic consequence from ingestion of asbestos. NIEHS has the responsibility for effecting these protocols. One aspect of the study is to determine if asbestos can act as a co-carcinogen, specifically in the gastrointestinal tract. <u>1,2-Dimethylhydrazine (DMH)</u> and <u>methylazoxymethanol (MAM)</u> are known intestinal carcinogens, however, the dose required to produce a relatively low incidence (15 + 5%) of <u>colon cancer</u> is not known. The objective of this study is to derive this information and in addition, to critically evaluate any neoplasm produced as to location, size, and morphology.		

PROJECT DESCRIPTION

METHODS EMPLOYED: Five week old male and female Fischer (CDF) rats were given various amounts (30, 15, 7.5, 4, 1.0 and 0.2 mg/kg) of either DMH or MAM via gavage. They were dosed every other week for 8 weeks (5 doses). Body weight gain was monitored. The study was terminated at 12 to 14 months after the first dose. The incidence of neoplasia and latent period was recorded. Tumors were recorded as to their location, number, size and morphology.

MAJOR FINDINGS AND PROPOSED COURSE: There was a small but significant dose related decrease in weight gain in the 30 mg/kg animals (both compounds). The findings suggest that the dose of both compounds required to produce a 15-25% incidence of colon neoplasia is 15 mg/kg in female and 7.5 mg/kg in male rats. However, there were more tumors in other locations with MAM. For this reason, DMH was considered a better model for use in the asbestos study. Project has been completed.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Colon cancer is the 2nd leading form of neoplasia in men and 3rd in women. Various studies have indicated that it is probably environmentally related. Suggestions have been made that asbestos may play a role in the causation. The development of a method which would yield a relatively low incidence would serve as a useful model for the study of this important disease.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30079-01 EBCB																																				
PERIOD COVERED October 1, 1977 to September 30, 1978																																						
TITLE OF PROJECT (80 characters or less) Acute and Subacute Toxicity of Firemaster FF-1 and 2,4,5,2',4',5'-Hexabromobiphenyl in the Rat and Mouse																																						
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0"> <tr> <td>PI:</td> <td>J.A. Moore</td> <td>Supervisory Medical Officer</td> <td>EBCB NIEHS</td> </tr> <tr> <td></td> <td>E.E. McConnell</td> <td>Veterinary Pathologist</td> <td>EBCB NIEHS</td> </tr> <tr> <td></td> <td>B.N. Gupta</td> <td>Veterinary Pathologist</td> <td>EBCB NIEHS</td> </tr> <tr> <td></td> <td>J.A. Goldstein</td> <td>Pharmacologist</td> <td>EBCB NIEHS</td> </tr> <tr> <td></td> <td>P. Linko</td> <td>Biological Laboratory Technician</td> <td>EBCB NIEHS</td> </tr> <tr> <td></td> <td>M.W. Harris</td> <td>Biological Laboratory Technician</td> <td>EBCB NIEHS</td> </tr> <tr> <td></td> <td>J.D. Allen</td> <td>Biological Laboratory Technician</td> <td>EBCB NIEHS</td> </tr> <tr> <td></td> <td>D.L. Myers</td> <td>Biological Laboratory Technician</td> <td>EBCB NIEHS</td> </tr> <tr> <td></td> <td>R.E. Wilson</td> <td>Biological Laboratory Technician</td> <td>EBCB NIEHS</td> </tr> </table>			PI:	J.A. Moore	Supervisory Medical Officer	EBCB NIEHS		E.E. McConnell	Veterinary Pathologist	EBCB NIEHS		B.N. Gupta	Veterinary Pathologist	EBCB NIEHS		J.A. Goldstein	Pharmacologist	EBCB NIEHS		P. Linko	Biological Laboratory Technician	EBCB NIEHS		M.W. Harris	Biological Laboratory Technician	EBCB NIEHS		J.D. Allen	Biological Laboratory Technician	EBCB NIEHS		D.L. Myers	Biological Laboratory Technician	EBCB NIEHS		R.E. Wilson	Biological Laboratory Technician	EBCB NIEHS
PI:	J.A. Moore	Supervisory Medical Officer	EBCB NIEHS																																			
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	D.L. Myers	Biological Laboratory Technician	EBCB NIEHS																																			
	R.E. Wilson	Biological Laboratory Technician	EBCB NIEHS																																			
COOPERATING UNITS (if any) J. Weaver, North Carolina State University, Raleigh, North Carolina																																						
LAB/BRANCH Environmental Biology and Chemistry Branch																																						
SECTION Comparative Pathology Section																																						
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																																						
TOTAL MANYEARS: 2.7	PROFESSIONAL: 0.9	OTHER: 1.8																																				
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINDRS ☐ (a2) INTERVIEWS																																						
SUMMARY OF WORK (200 words or less - underline keywords) Toxicity studies with Firemaster FF-1 (mixture of <u>polybrominated biphenyls</u>) and pure 2,4,5,2',4',5'-hexabromobiphenyl (<u>HBB</u>) in <u>rats</u> and <u>mice</u> were conducted in two steps: (a) rats and mice of both sexes were given either compound via gavage (daily 5x/wk for 1 month) and were examined 15, 30, 45, 60 and 120 days post treatment; (b) Firemaster FF-1 only, was then given to rats and mice at various concentrations (total 125 daily doses, 5x/wk for 6 months). The following parameters were studied: (a) food consumption; (b) body weight gain; (c) hematology; (d) clinical pathology; (e) anatomic pathology; (f) thyroid function; (g) urinary and tissue porphyrins; and (h) liver drug metabolizing enzymes.																																						

PROJECT DESCRIPTION

OBJECTIVE: The purpose of these toxicological studies were to determine the effect of short and intermediate exposure of Firemaster FF-1 and its major component, 2,4,5,2',4',5'-hexabromobiphenyl in the rat and mouse.

METHODS EMPLOYED: (a) Firemaster FF-1 (Lot No. 1312 FT) or 2,4,5,2',4',5'-hexabromobiphenyl (HBB) were given to rats and mice via gavage (5x/wk for 1 month) and were examined at 15, 30, 45, 60 and 120 days following exposure. Tissue bromine levels were measured by neutron activation (NA), T_3 and T_4 uptake by radio immune assay (b) Firemaster was given similarly during a period of 180 days and the rats and mice were then examined. Representative animals from the 6 month exposure will be held for 18 months to observe the long term effect of Firemaster FF-1 exposure.

MAJOR FINDINGS AND PROPOSED COURSE: Although there was no significant difference in food consumption between treated and control animals, there was significant decrease in body weight gain at the higher doses of FF-1. Firemaster FF-1 was porphyrinogenic in mice only in the short term study. The liver was the major target organ followed by lymphoid organs such as thymus and spleen. The liver was markedly enlarged with accentuation of lobular markings. The severity of changes in liver was dose dependent. Microscopically, there were slight to moderate swelling of hepatocytes, fatty metamorphosis, single cell necrosis and granulomatous foci in the liver. There was slight involution of the thymus. Hematologic and clinical chemistry abnormalities were more apparant with FF-1 than HCB. FF-1 was a mixed inducer of drug-metabolizing enzymes, inducing Arylhydrocarbon hydroxylase (AHH) N-dimethylase (ND), cytochrome P-450, and altering its spectral characteristics. The effects of HBB were different than those of FF-1 regardless of dose. HBB was a better inducer of ND but did not induce AHH. It was a pure phenobartital type inducer. The accumulation of HBB and FF-1 in liver and fat were similar when corrected for dose. During the recovery period, neither HBB nor FF-1 disappeared from fat, but hepatic levels dropped by 75%. Recovery of liver enzymes was not complete but resembled tissue levels. Many of the parameters of the 30 day study are still being evaluated and the 6 month study of Firemaster FF-1 with rats and mice is still in progress as of 1 April 1978. Further study to determine the long term effect of Firemaster FF-1 in laboratory animals will be conducted.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The fire retardant, Firemaster FF-1, a mixture of polybrominated biphenyls and possibly other toxic compounds, was responsible for widespread environmental contamination, animal loss and human illness in Michigan during 1973-1974. Long-term effects in animals and possibly humans are still apparant because of the extremely long biological half-life and persistence in the environment. Because of this it is important to evaluate in depth the short and long term toxicologic effects of the compound in laboratory animals in order to project the possible effects in man. The above study was carried out as part of the Toxicologic Bioassay Program as a prelude to a standard carcinogen bioassay.

PROJECT DESCRIPTION

METHODS EMPLOYED: Six inch, 13 gauge stainless steel feeding needles were shaped to the curvature of the rabbit pharynx. The needles were passed over the base of the tongue into the esophagus.

MAJOR FINDINGS AND PROPOSED COURSE: This method of gastric intubation facilitated administering precise doses of liquid to rabbits without the danger of accidentally entering the trachea.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Experimental drugs and other liquids may be administered to rabbits with minimal discomfort and danger to the animal. This technique is employed by many investigators in toxicology at NIEHS and other research facilities. The project has been completed.

PUBLICATIONS

Feldman, D.B.: Simplified Gastric Intubation in the Rabbit. Lab. An. Sci. 27: 1037, 1977

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: Statistical analysis, and preparation of a manuscript were completed this year. Results indicate strongly that all of the effects of HCB (porphyria, induction, hepatic, lung and skin changes) are due to HCB, not its contaminants. Work on the effects on drug metabolism will continue.

PUBLICATIONS

Goldstein, J.A., Friesen, M., Scotti, T.M., Hickman, P., Hass, J.R., and Bergman, H. Assessment of the Contribution of Chlorinated Dibenzo-p-dioxins and Dibenzofurans to Hexachlorobenzene-Induced Toxicity, Porphyria, Changes in Mixed Function Oxygenases and Histopathological Changes. Tox. Appl. Pharmacol. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30084-01 EBCB												
PERIOD COVERED October 1, 1977 to September 30, 1978														
TITLE OF PROJECT (80 characters or less) Dose-response of Drug-metabolizing Enzymes to Polybrominated Biphenyls and Naphthalenes														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 35%;">J.A. Goldstein</td> <td style="width: 40%;">Pharmacologist</td> <td style="width: 15%;">EBCB NIEHS</td> </tr> <tr> <td>OTHER:</td> <td>P. Linko</td> <td>Biological Laboratory Technician</td> <td>EBCB NIEHS</td> </tr> <tr> <td></td> <td>J.D. McKinney</td> <td>Supervisory Research Chemist</td> <td>EBCB NIEHS</td> </tr> </table>			PI:	J.A. Goldstein	Pharmacologist	EBCB NIEHS	OTHER:	P. Linko	Biological Laboratory Technician	EBCB NIEHS		J.D. McKinney	Supervisory Research Chemist	EBCB NIEHS
PI:	J.A. Goldstein	Pharmacologist	EBCB NIEHS											
OTHER:	P. Linko	Biological Laboratory Technician	EBCB NIEHS											
	J.D. McKinney	Supervisory Research Chemist	EBCB NIEHS											
COOPERATING UNITS (if any) B. Gupta, Comparative Pathology L. Levy, Chemistry J. Weaver, NCSU														
LAB/BRANCH Environmental Biology and Chemistry Branch														
SECTION														
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709														
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.3	OTHER: 0.3												
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords) The <u>polybrominated biphenyls</u> (PBBs) are inducers of hepatic <u>mixed-function oxidases</u> . The purpose of this study is to compare the structure-activity relationships of <u>2,4,5,2',4',5'-hexabromobiphenyl</u> (2,3,4-HBB), and other components of the commercial polybrominated biphenyl mixture, Firemaster BP-6 as inducers of <u>cytochromes P-450</u> and <u>P₄₅₀</u> and to relate these effects to hepatic toxicity.														

PROJECT DESCRIPTION

METHODS EMPLOYED: Female rats were injected i.p. with various equimolar doses (1.6-1000 μ mol/kg) of 2,4,5-HBB, 2,3,6,7-tetrabromonaphthalene (TBN) and the commercial PBB mixture (FF-1). Four days later, they were sacrificed; body weight gain, liver weight, and liver benzo(a)pyrene hydroxylase (AHH), aminopyrine N-demethylase (ND), cytochrome P-450 and ethyl isocyanide (EtNC) ratios determined. Liver samples were taken for histology.

MAJOR FINDINGS AND PROPOSED COURSE: The commercial mixture, FF-1, is a mixed inducer, intermediate between phenobarbital and 3-methylchloranthrene as an inducer. It induces AHH, ND, P-450 and alters the EtNC ratios. 2,4,5-HBB is a phenobarbital type inducer, inducing ND and P-450 but producing little change in AHH or the EtNC ratios. TBN is a 3-methylchloranthrene type inducer, inducing AHH and P-448 but not ND. The potency of TBN is approximately equipotent to that of FF-1, and much less than that of the structurally related 2,3,7,8-tetrachlorodibenzofuran or tetrachlorodibenzodioxin which have been examined previously. When TBN is administered simultaneously with 2,4,5-HBB at a concentration equal to that of the total brominated naphthalenes found in 2,4,5-HBB, there is no perceptible synergism, i.e., naphthalenes do not appear to account for the P-448 induction seen with FF-1. Livers are being evaluated histologically. Other isomers including the penta- and hexa-naphthalenes may be evaluated as these become available.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: There has been considerable human exposure to PBBs and PCBs (polychlorinated biphenyls). These experiments were designed to analyze the structure-activity relationships of PBBs. Toxicity appears to correlate well with structure-activity relationships. In particular, those compounds which induce P-448 are the most toxic although it is not yet clear as to whether the two classes of hepatic inducers also produce different spectra of toxicity.

PUBLICATIONS

Goldstein, J.A. and Hickman, P. Comparison of a Commercial Polybrominated Mixture (Firemaster BP-6) with 2,4,5,2',4',5'-Hexabromobiphenyl and a Tetrabromonaphthalene as Inducers of Hepatic Mixed-Function Oxidases. Toxicol Appl. Pharmacol., Seventeenth Annual Meeting of Toxicology, San Francisco, California, March 12-16, 1978. (Abstract) (In press).

PROJECT NUMBER (Do NOT use this space)

HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

July 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Tumorigenic Potential of NO₂ Inhalation in Small Animals Exposed to
Heterocyclic AminesNAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECTPI: E.W. Van Stee
G.A. BoormanPhysiologist
Comparative PathologistEBCB, NIEHS
EBCB, NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Environmental Biology and Chemistry Branch

SECTION

Inhalation Toxicology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:

1.0

PROFESSIONAL:

.5

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS☐ (b) HUMAN TISSUES☐ (c) NEITHER☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Random bred, male Fisher 344 rats, CD-1 mice, and golden Syrian hamsters are being exposed to 1 ppm of NO₂ for 6 hr/day, 5 da/wk for 30 weeks. Some of the animals are also receiving 0.1% morpholine in deionized water as their sole source of drinking water. The mice and hamsters will be held for 10 wk and the rats for 50 wk after the 30 wk NO₂ exposure period during which time all animals will receive deionized water. Animals will be killed for histopathological evaluation following the postexposure holding period.

Similar studies will be conducted in which the animals are exposed to morpholine by inhalation. Future studies will include other heterocyclic amines, e.g. peperazine.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30085-01 EBCB																				
PERIOD COVERED October 1, 1977 to September 30, 1978																						
TITLE OF PROJECT (80 characters or less) Early Detection of Lung Injury																						
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																						
<table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 40%;">E. G. Tombropoulos</td> <td style="width: 40%;">Research Chemist</td> <td style="width: 10%; text-align: right;">EBCB NIEHS</td> </tr> <tr> <td></td> <td>E. W. Van Stee</td> <td>Physiologist</td> <td style="text-align: right;">EBCB NIEHS</td> </tr> <tr> <td>OTHER:</td> <td>W. Gibson</td> <td>Chemist</td> <td style="text-align: right;">EBCB NIEHS</td> </tr> <tr> <td></td> <td>M. Riley</td> <td>Biological Laboratory Technician</td> <td style="text-align: right;">EBCB NIEHS</td> </tr> <tr> <td></td> <td>J. Schroeder</td> <td>Biological Laboratory Technician</td> <td style="text-align: right;">EBCB NIEHS</td> </tr> </table>			PI:	E. G. Tombropoulos	Research Chemist	EBCB NIEHS		E. W. Van Stee	Physiologist	EBCB NIEHS	OTHER:	W. Gibson	Chemist	EBCB NIEHS		M. Riley	Biological Laboratory Technician	EBCB NIEHS		J. Schroeder	Biological Laboratory Technician	EBCB NIEHS
PI:	E. G. Tombropoulos	Research Chemist	EBCB NIEHS																			
	E. W. Van Stee	Physiologist	EBCB NIEHS																			
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	J. Schroeder	Biological Laboratory Technician	EBCB NIEHS																			
COOPERATING UNITS (if any) None																						
LAB/BRANCH Environmental Biology and Chemistry Branch																						
SECTION Inhalation Toxicology Section																						
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																						
TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.3	OTHER: 0.3																				
CHECK APPROPRIATE BOX(ES)																						
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER																						
☐ (a1) MINORS ☐ (a2) INTERVIEWS																						
SUMMARY OF WORK (200 words or less - underline keywords) <u>Pulmonary lavage</u> is easily obtainable and can be taken at various time intervals from the same animal (large such as dogs) without killing the animal. Pulmonary lavage contains lipoproteins, lipids, proteins, cells including macrophages and enzymes. The majority of these components are synthesized by the lung but some come via the pulmonary circulation, and some originate from bone marrow such as the macrophages. The alveolar macrophages are vital entities of the mammalian immune mechanism (in the latter, capacity macrophages depend upon plasma membrane activity for migration recognition, activation, phagocytosis and attachment to substrate tissue). Thus lavage can be used as a probe for early detection of lung damage, and of lung immune mechanism.																						

PROJECT DESCRIPTION

METHODS EMPLOYED: Rats and rabbits were exposed to 250 ppm morpholine vapor for 6 hrs per day. The animals were exposed for 27 days in whole body exposure chambers. Control animals were placed in similar chambers for the same duration as the morpholine exposed.

At the end of the experiment both groups, the morpholine and sham exposed animals were divided into two subgroups; the one subgroup was killed immediately after the last exposure and the other subgroup two days later. Lung lavages were obtained, centrifuged and separated into supernatant and cellular material (macrophages). Enzymatic activities were measured in both the supernatant and the macrophage fractions after their disruption by sonication. The enzymes measured were: acid phosphatase, alkaline phosphatase, and β -N-Acetylglucosaminidase. An aliquot of non-disrupted macrophages was assayed for the binding of wheat germ agglutinin. A part of lung tissue was used for morpholine concentration analysis. Another part of lung tissue was homogenized and subfractionated into mitochondrial and microsomal fraction for the assay of aminooxidase and the P-450.

MAJOR FINDINGS AND PROPOSED COURSE: At the end of the exposure the morpholine concentration in the lung was: female rats-73.60 $\mu\text{g/gm}$ tissue; female rabbit-146.23 $\mu\text{g/gm}$ tissue; male rats-42.8 $\mu\text{g/gm}$ tissue; male rabbit-99.35 $\mu\text{g/gm}$ tissue. The group of animals killed immediately after exposure showed the following differences as compared to sham exposed animals. (1) β -N-Acetylglucosaminidase and acid phosphatase were elevated in both the supernatant and macrophage fraction of lung lavages. Alkaline phosphatase was elevated only in the cellular debris not associated with lung macrophages. (2) A higher percentage of alveolar macrophages were dead in the group of morpholine exposed compared to the sham exposed animals. (3) The binding of wheat germ agglutinin per alive or total macrophage was reduced in morpholine exposed animals. (4) A reduction of O_2 uptake of lung slices was observed in the morpholine exposed animals. (5) P-450 levels of lung microsomes were lower in the morpholine exposed group as compared to the controls. Two days postexposure, there were no differences between the morpholine and sham exposed animals except that the β -N-Acetylglucosaminidase was still elevated in the macrophage fraction and the P-450 reduced in the microsomal fraction of the lung. The morpholine levels for the rat were 6 $\mu\text{g/gm}$ tissue for the females and 3.74 $\mu\text{g/gm}$ for the males. The male rabbit was 2.06 $\mu\text{g/gm}$ tissue,

The elevated β -N-Acetylglucosaminidase and the decrease of binding by lung macrophages of wheat germ agglutinin which is specific for β -N-Acetylglucosamine agree very well. The high percentage of dead macrophages, the alterations of its surface membrane, and the reduction of microsomal P-450 indicates that in animals exposed to morpholine the immune and detoxifying mechanism of the lung is impaired for at least two days. During the next experiments, histopathological observations will have to be made to see what the biochemical observations of the lung lavage represent. Although our observations on alterations of membranes such

as the macrophage or epithelial cells (elevated alkaline phosphatase not associated with macrophage) agree with the findings of Dr. Lam using higher levels of morpholine; lung lavage to be used as a prognostic tool has to be correlated with pathological observation during the experiment or shortly following it.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

A great need exists for the development of indices of early toxic injury to the lung. The lung is a central organ in matters related to inhalation toxicology. The lung serves the function of portal of entry, site of biotransformation, and an organ of elimination for many compounds. Soluble compounds to which the organism is exposed by inhalation may or may not have a deleterious effect on the lung itself. The lung may constitute the organ through which the parent compound and/or its products of biotransformation are introduced into the organism with the result of the production of tissue injury to the lung itself or other organs of the body. The objective of this project is the search for evidence of early toxic injury that may shed light on subsequent events.

PUBLICATIONS

Hubbard, W.D., Prosser, A.R., Sheppard, A.J., Newkirk, D.R., Osgood, T., Tombropoulos, E.G. and Johns, S.T. Individual lipids and proximate analysis of various foods. I. French fried potatoes from ten chain restaurants. Journal of Agric. and Food Chem. Vol. 25: 280-281, 1977.

Hook, G.E.R., Gilmore, L.B., Tombropoulos, E.G. and Fabro, S.E. Fetal lung lamellar bodies in human amniotic fluid. Am. Rv. of Resp. Dis. Vol. 117: 541-550, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30086-01 EBCB						
PERIOD COVERED October 1, 1977 to September 30, 1978								
TITLE OF PROJECT (80 characters or less) Toxicity and LD ₅₀ Determination of Firemaster FF-1 in the Rat								
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%;"> <tr> <td style="width: 33%;">PI: B.N. Gupta</td> <td style="width: 33%;">Comparative Pathologist</td> <td style="width: 33%;">EBCB NIEHS</td> </tr> <tr> <td>Other: J.A. Moore</td> <td>Supervisory Veterinary Medical Officer</td> <td>EBCB NIEHS</td> </tr> </table>			PI: B.N. Gupta	Comparative Pathologist	EBCB NIEHS	Other: J.A. Moore	Supervisory Veterinary Medical Officer	EBCB NIEHS
PI: B.N. Gupta	Comparative Pathologist	EBCB NIEHS						
Other: J.A. Moore	Supervisory Veterinary Medical Officer	EBCB NIEHS						
COOPERATING UNITS (if any) None								
LAB/BRANCH Environmental Biology and Chemistry Branch								
SECTION Comparative Pathology								
INSTITUTE AND LOCATION NIH, NIEHS, Research Triangle Park, North Carolina 27709								
TOTAL MANYEARS: 1.5	PROFESSIONAL: 0.25	OTHER: 0.25						
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS								
SUMMARY OF WORK (200 words or less - underline keywords) When given orally for 1 month (5 days/week, 22 total doses) to Fischer 344 rats, the LD ₅₀ (90-day-observation) was estimated to be 65 mg/kg/day (total 1.43 gm/kg) for the female rat and 149 mg/kg/day (total 3.28 gm/kg) for the male. All female rats given 22 doses @ 100 mg/kg/day (total 2.20 gm/kg) died between 41 and 53 days while only 38% of the males died between 50 and 73 days. Pathologic changes in treated rats were enlargement of the liver with accentuation of the hepatic lobular markings and atrophy of thymus and spleen. Microscopically, hepatic changes were characterized by congestion, fatty infiltration, and multifocal liquefactive necrosis. Male rats given 100 mg/kg/day and dying after 90 days had subacute to chronic hepatitis with marked focal proliferation of bile ducts.								

PROJECT DESCRIPTION

OBJECTIVE: To determine the LD₅₀ and toxicity of a fire retardant, Firemaster FF-1 (polybrominated biphenyl).

METHODS EMPLOYED: Seven to eight weeks old, Fischer 344/N rats of both sexes were given Firemaster FF-1 orally @ 0 (control), 30, 100 and 1,000 mg/kg body weight. The Firemaster FF-1 (Lot # 1312FT) contained a mixture of polybrominated biphenyl and 2% calcium trisilicate. The Firemaster FF-1 was mixed in corn oil and was given daily, 5 days/week for 4 1/2 weeks, a total of 22 doses. All rats were observed for a total of 120 days from the 1st day of treatment.

Food consumption and body weight were recorded once a week. Dead rats were necropsied, and tissues were fixed in buffered neutral 10% formalin for histopathologic examination. Bones, teeth, liver and other organs were grossly examined under long wavelength (366 nm) ultraviolet light for the presence of red fluorescence as an indication of excess porphyrin accumulation.

MAJOR FINDINGS AND PROPOSED COURSE: The LD₅₀ (90-day observation) of Firemaster FF-1 was estimated to be 65 mg/kg/day (total 1.43 gm/kg) for the female rat and 149 mg/kg/day (total 3.28 gm/kg) for the male rats. All female rats given 22 doses @ 100 mg/kg/day (total 2.2 gm/kg) died between 41 and 53 days while only 38% of the males died between 50 and 73 days.

The rats which became moribund or died appeared dehydrated, emaciated and lacked subcutaneous and visceral adipose tissue. The liver was markedly enlarged with marked accentuation of lobular markings and appeared darker in color. The thymus was markedly involuted and only a small remnant of gelatinous connective tissue element was observed in place of the thymus.

Microscopically, the liver had multifocal coagulative or liquefactive necrosis, along with fibrosis and presence of inflammatory exudate. Male rats dying given 100 mg/kg/day (22 doses) and dying between 50 and 100 days had either diffuse or focal proliferation of bile ducts. In focal biliary proliferation, the nuclei were hyperchromic with occasional mitoses. There were necrosis, hyperplasia, mitoses and squamous metaplasia with keratinization of the epithelial lining of ductus deferens of male rats given 100 mg/kg.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Firemaster FF-1, a polybrominated biphenyl fire retardant, was responsible for the widespread environmental contamination and animal loss in Michigan during 1973 and 1974. Although the contamination of feed with Firemaster FF-1 was an accident, it resulted in widespread and high level contamination of livestock and poultry feed. The residents of Michigan also were exposed by consuming the tainted meat, dairy products, pork and poultry. In lower doses, Firemaster FF-1 had a somewhat delayed and persistent hepatotoxic effect, and was more lethal than previously reported and may possibly be a carcinogen.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80020-06 EBCB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Changes in Mammalian Pulmonary Function Produced by Inhaled Environmental Agents		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	J. Takezawa H.F. Lam E.W. Van Stee	Visiting Fellow Visiting Fellow Physiologist
OTHER:	J. Speas	Biological Aid
EBCB NIEHS EBCB NIEHS EBCB NIEHS EBCB NIEHS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Environmental Biology and Chemistry Branch		
SECTION Inhalation Toxicology Section		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MANYEARS: 1.4	PROFESSIONAL: 1.1	OTHER: 0.3
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Changes in <u>pulmonary function</u> resulting from <u>inhalation of airborne pollutants</u> are being estimated using a whole <u>body pressure plethysmograph</u> constructed for use with small animals. Both acute and chronic exposure to oxidants, singly and in combination with other compounds result in functional changes. Changes in <u>pulmonary mechanics</u> (e.g. <u>resistance</u>, <u>compliance</u>), <u>spirometry</u> (e.g. lung volumes, frequency of breathing), <u>heart rate</u>, and <u>pulmonary morphology</u> will be estimated.		

PROJECT DESCRIPTION

METHODS EMPLOYED: We have established the methods to assess the changes in pulmonary function induced by environmental toxicants. Our methods include single breath diffusing capacity for carbon monoxide in the lung (D_{LCO}), neon dilution to measure lung volumes such as residual volume (RV), functional residual capacity (FRC), and total lung capacity (TLC), and Amdur method to measure dynamic compliance and resistance in small laboratory animals.

MAJOR FINDINGS AND PROPOSED COURSE: D_{LCO} and lung volumes of normal mammals from hamsters to rabbits have been measured (1). Morpholine was selected as the first compound to evaluate the sensitivity of our method to detect the damage in the lung. Pulmonary function testing was often more sensitive than light microscopical examination for detecting lung effects of inhaled toxicants. We have found that acute (2000 ppm) and subacute (450 ppm) exposure of morpholine to male rats induced changes in pulmonary function which suggested obstructive lung defect (2).

We are now interested in CO_2 response curve of neonatal guinea pigs which have been exposed to 10% of oxygen in air post partum. We suspect that in a certain period neonates set down their threshold to hypoxemia and hypercapnia according to the ambient O_2 and CO_2 conditions. This mechanism could be of significance in infant sudden death syndrome.

Since D_{LCO} and lung volumes of animals subjected to Paraquat administration have not been reported, we selected this compound and related ones to clarify the physiological or clinical picture of intoxication by these compounds.

We are also interested in assessing the changes in lung function in animals exposed to a highly alkaline vapor of similar pH to morpholine.

Pulmonary function testing will be deleted from the program of the Inhalation Toxicology Section effective June, 1978.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Changes in pulmonary function of animals after exposure to inhaled environmental chemicals are known, in many cases, to be associated with cellular damage and morphologic changes. Inhalation exposure to relatively high concentrations of morpholine vapors (250-450 ppm) for many hours has been demonstrated to induce functional changes in small laboratory animals that have the potential for being correlated with structural changes. The returns are incomplete at this writing but it is hoped that this aspect of the Section program will be terminated with a reasonable complete structure-function relationship established for the pulmonary toxicity of inhaled morpholine.

PUBLICATIONS

Takezawa, J., Miller, J.M., and O'Neil, J.J.: Lung volume and the single breath diffusing capacity for carbon monoxide measured in small laboratory mammals. Am. Rev. Resp. Dis. 117:---, 1978.

Takezawa, J., and Lam, H.F. (Sponsor: Van Stee, E.W.): Toxic effect of morpholine on rats lung. Fed. Pro. 37: 247, 1978.

RESEARCH SERVICES BRANCH

RESEARCH SERVICES BRANCH Summary Statement

The Research Services Branch plans and coordinates new facilities for the Institute as well as operates and maintains existing facilities. It also provides a variety of research support functions such as laboratory instrumentation services, glassware and media preparation, arts, graphics, and photography.

Renovation of existing facilities has often lagged behind demand, principally due to significant turnover in personnel and in the decision to prioritize renovations that relate to laboratory and worker safety. The permanent site currently under construction is also increasing demands on the engineering personnel within the Research Services Branch as well as the administrative personnel within the Office of the Associate Director.

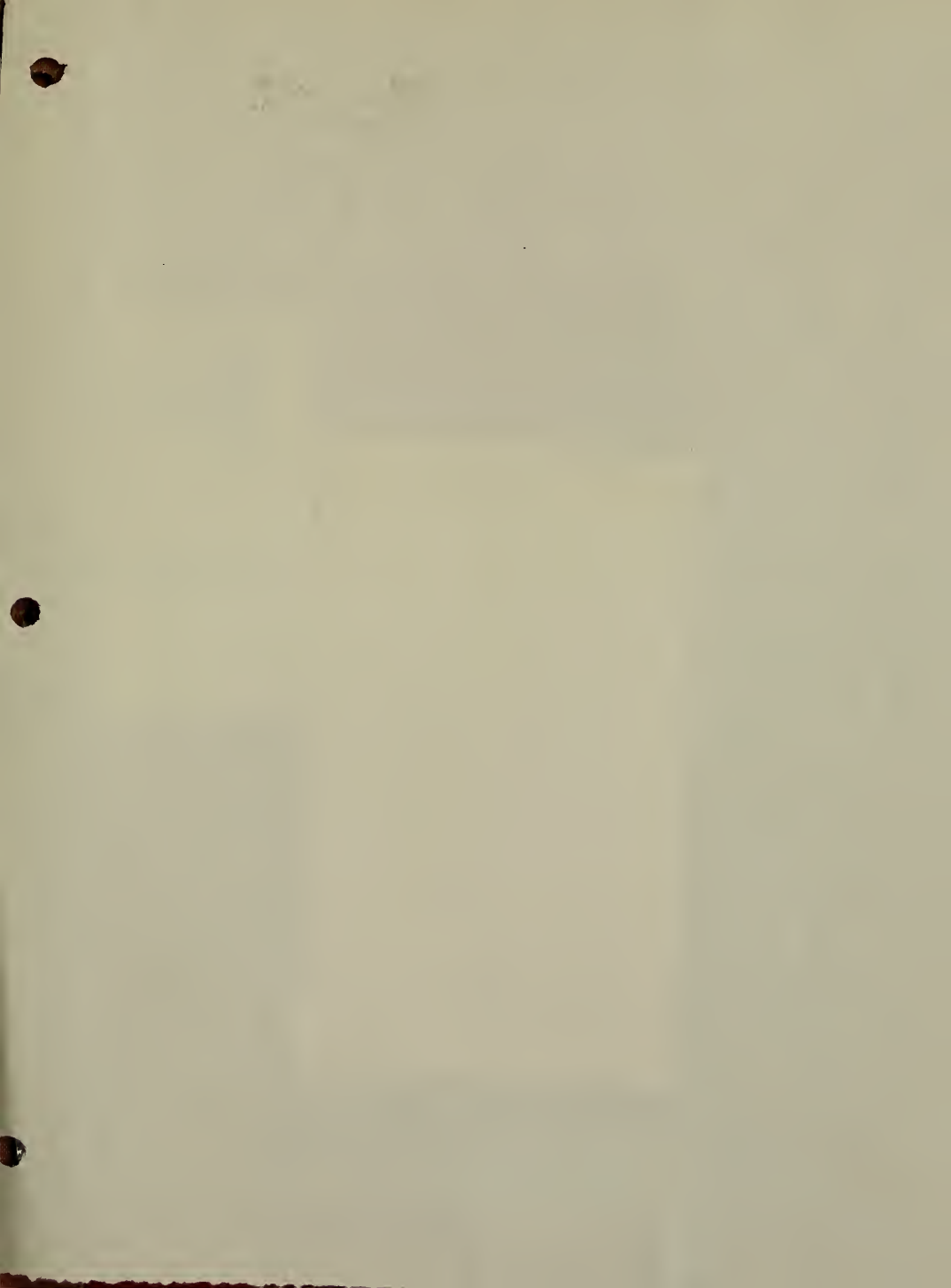
Facilities: NIEHS currently utilizes 208,072 square feet of leased space. The principal interim site provides 169,774 square feet in a complex of 19 buildings. The Institute has a staff housing capability in excess of 400. Facilities operation, maintenance, and alterations are carried out by the plant engineering staff. One of its major activities has been renovating laboratory and office space to meet specific program needs. An additional 7,000 net square feet of temporary office space is to be provided through the use of trailers that will be located on a site adjacent to the main interim complex.

Permanent NIEHS facilities are being constructed on a 509 acre tract of land that is in close proximity to the principal interim facilities. The facilities, designed for NIEHS requirements, were funded by a \$67 million Congressional appropriation. A construction management firm was selected in December 1976; construction contracts were subsequently awarded and initial construction begun in April 1977. Construction will be completed and Institute staff are to begin occupying the new facilities in 1980. NIEHS will continue to occupy the principal interim facilities. The "off site" leased facilities will not be retained once the permanent facilities are occupied.

The table below outlines the function and amount of space in both the interim and new facilities. Staffing capability is projected at approximately 800 for the new facility and 400 in the existing quarters.

Laboratory Services: Centralized glassware and media preparation services are provided to all laboratories. Glassware processing averages in excess of 60,000 pieces per month; a number of types of disposable glassware are also supplied. Media preparation averages 1500-2500 liters per month of a wide variety of types, including tissue culture and microbiological preparations.

Laboratory instrumentation services include design and fabrication of specialized instrumentation, fabrication of assorted laboratory paraphernalia, and repair and maintenance of laboratory equipment.



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